SUPPLEMENTARY INFORMATION
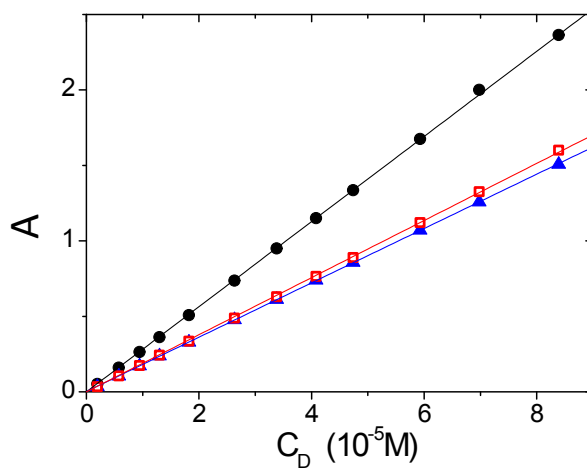

Figure. 1S Absorbance vs. concentration plots of TFT at different wavelengths. I = 0.11M, pH 7.0, T = 25°C, (▲) 385nm, (●) 412nm, (□) 435 nm.
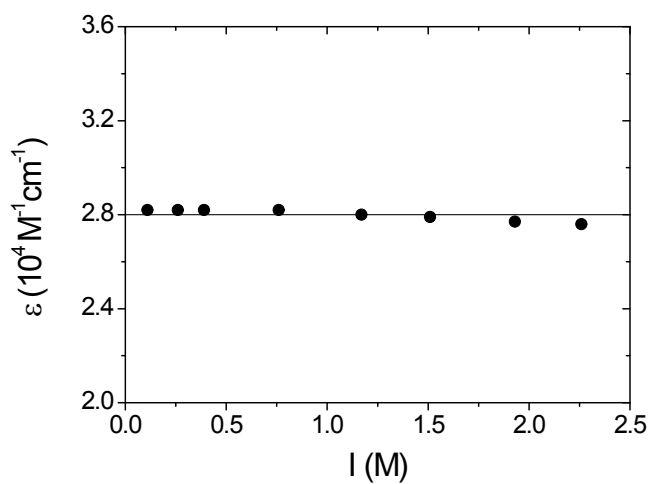

Figure 2S Molar extinction coefficient of TFT dependence on the salt content (I) of the medium (NaCl). $C_D = 2.9 \times 10^{-5}$ M, pH 7.0, $\lambda = 412$nm, T = 25°C.
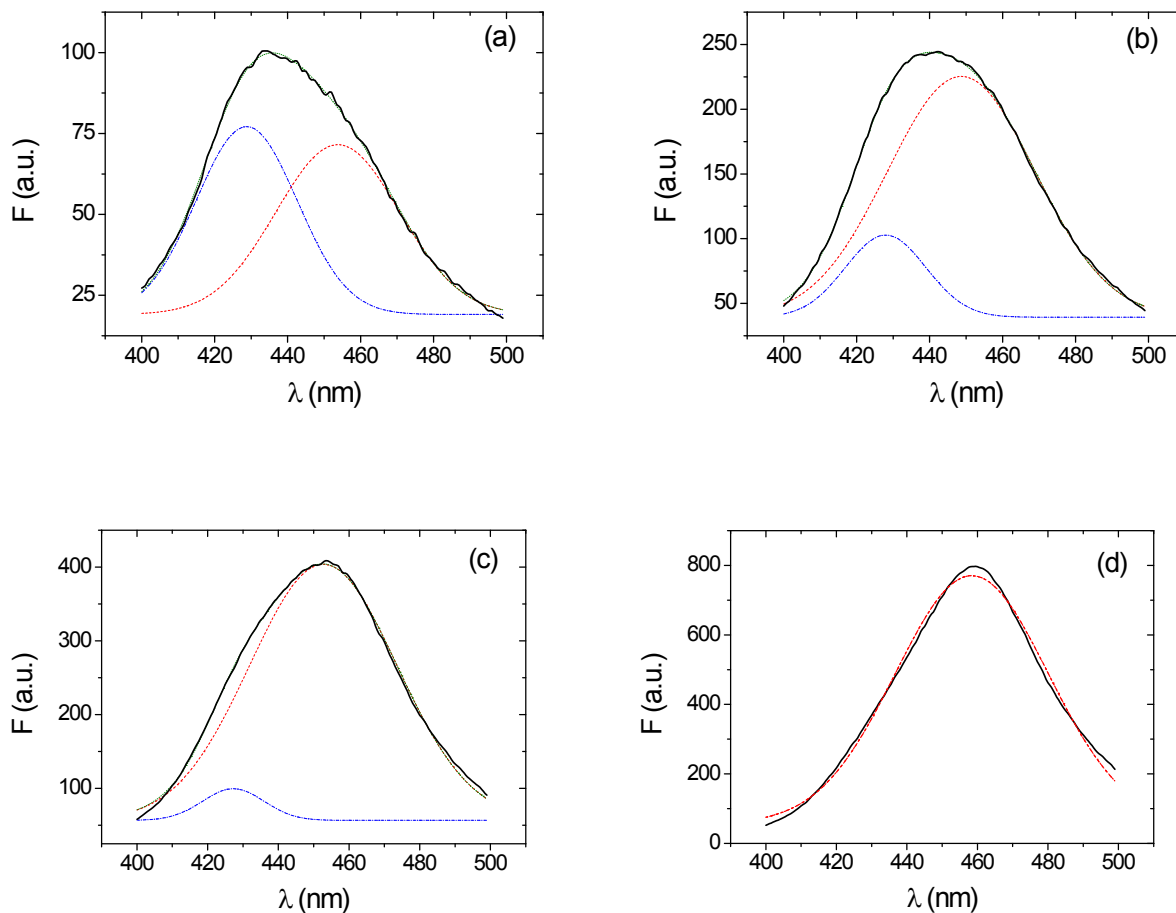
Figure 3S Two-component deconvolution of the fluorescence emission spectra of TFT at different dye concentrations, I = 0.11 M, pH 7.0, λ_{ex} = 340 nm, T = 25°C. (a) C_D = 1.44×10^{-6} M, $F(\lambda_{em1})/F(\lambda_{em2})$ = 0.9; (b) C_D = 7.19×10^{-6} M, $F(\lambda_{em1})/F(\lambda_{em2})$ = 2.9, (c) C_D = 3.56×10^{-5} M, $F(\lambda_{em1})/F(\lambda_{em2})$ = 8.1; (d) C_D = 7.06×10^{-5} M, $F(\lambda_{em1})/F(\lambda_{em2})$ indefinitely high; $F(\lambda_{em1})/F(\lambda_{em2})$ is the ratio of fluorescence intensities at the maxima of the two component emissions. (—) experimental; (- • -) first component; (---) second component; (•••) calculated sum (in the case of two components).
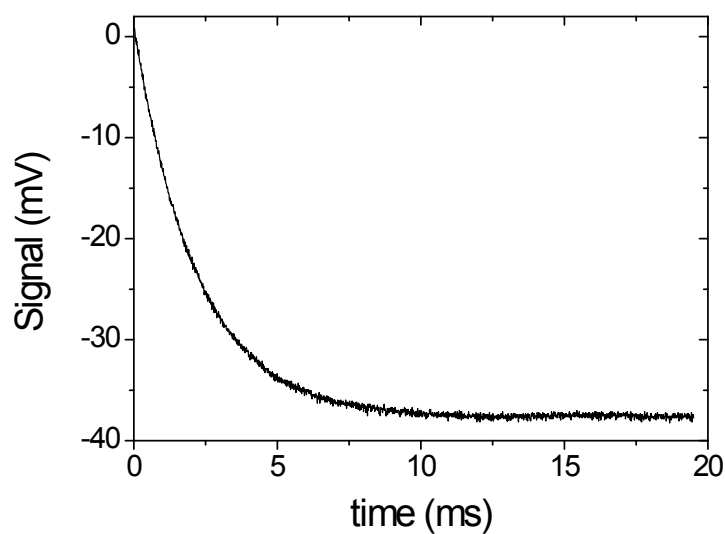
Figure 4S Example of kinetic trace recorded for a solution containing TFT alone and therefore demonstrating the presence of an auto-aggregation process. $C_D = 2.01 \times 10^{-4}$ M, I = 0.11 M, pH 7.0, λ = 450nm, T = 25°C.
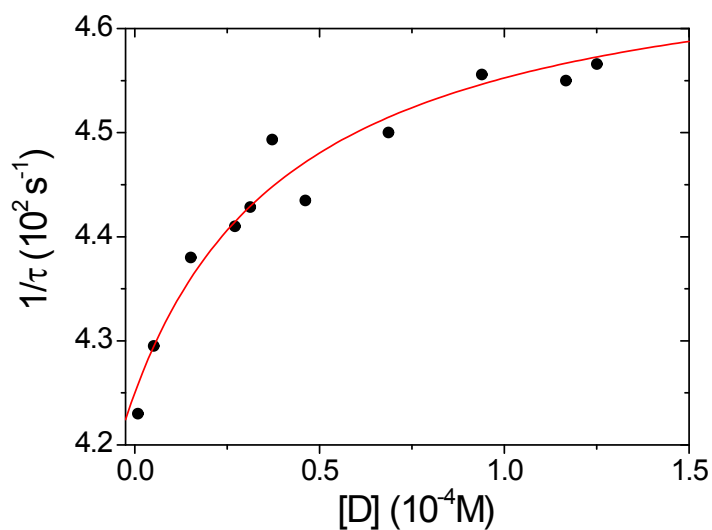

Figure 5S Plot of the reciprocal relaxation time ($1/\tau$) vs. TFT monomer concentration ([D]) analysed according to equation (4) (continuous line); I = 0.11 M, pH 7.0, T = 25 °C.

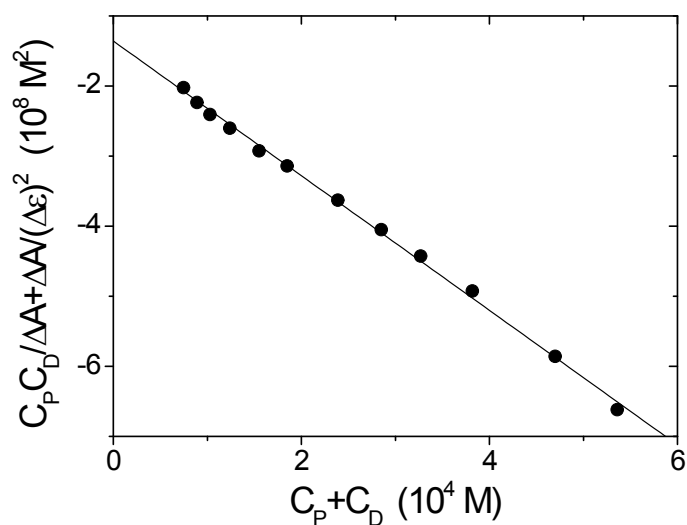**Figure 6S** Analysis of the data of the spectrophotometric titration of the TFT/DNA system according to equation (6) (straight line). $C_D = 5.2 \times 10^{-5}$ M, $C_P = 0$ to 5.1×10^{-4} M, I = 0.01 M, pH 7.0, T = 25 °C
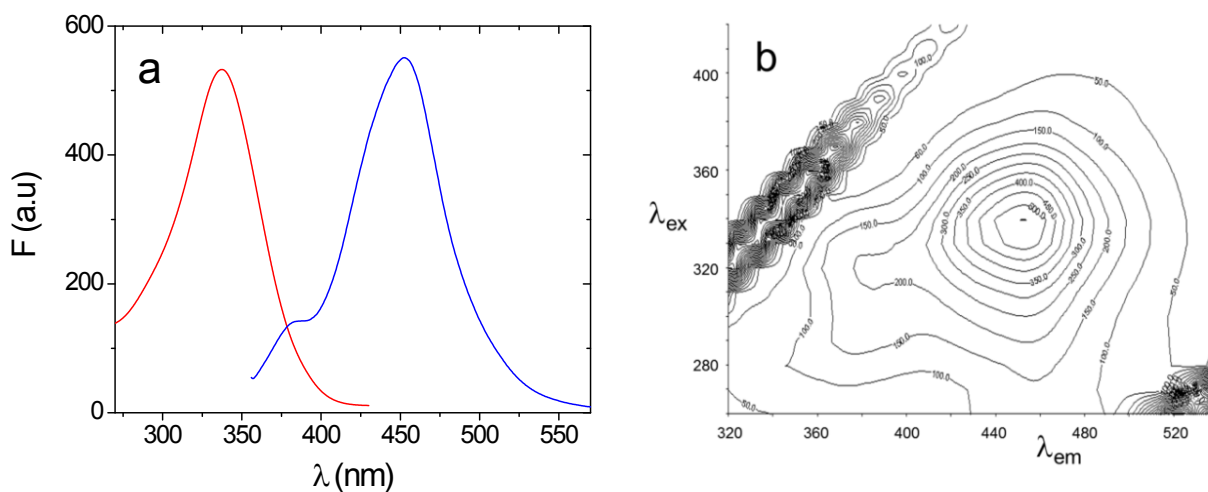
Figure 7S Excitation ($\lambda_{em} = 450$ nm) and emission ($\lambda_{exc} = 340$ nm) spectra (a) and 3D contour plot (b) of TFT. $C_D = 3.6 \times 10^{-5}$ M, I = 0.11 M, pH 7.0, T = 25 °C.
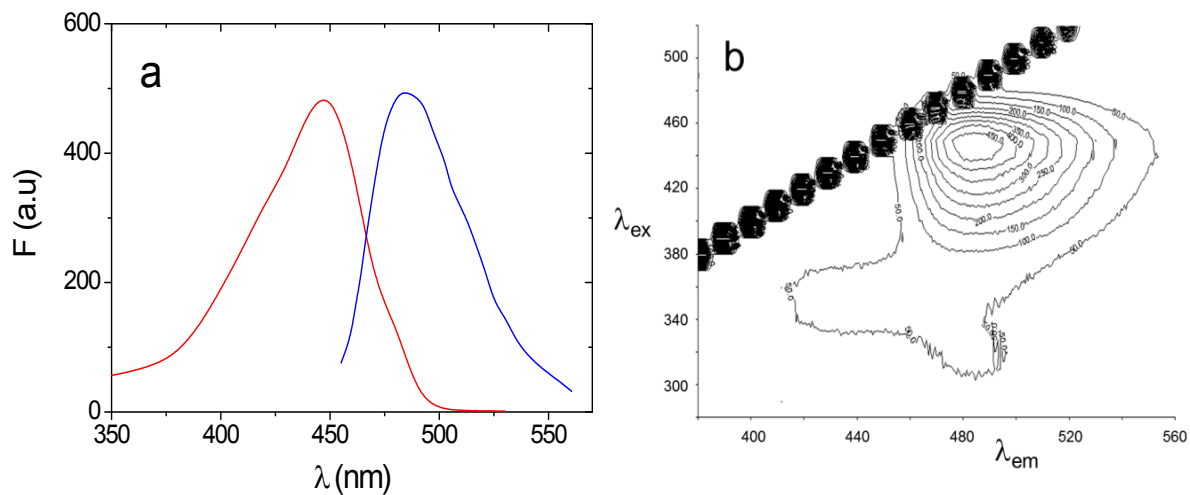
Figure 8S Excitation (λ_{em} = 484 nm) and emission (λ_{exc} = 450 nm) spectra (a) and 3D contour plot (b) of a TFT/DNA. C_D = 3.6×10^{-5} M, C_P = 2.5×10^{-4} M, I = 0.01 M, pH 7.0, T = 25 °C.
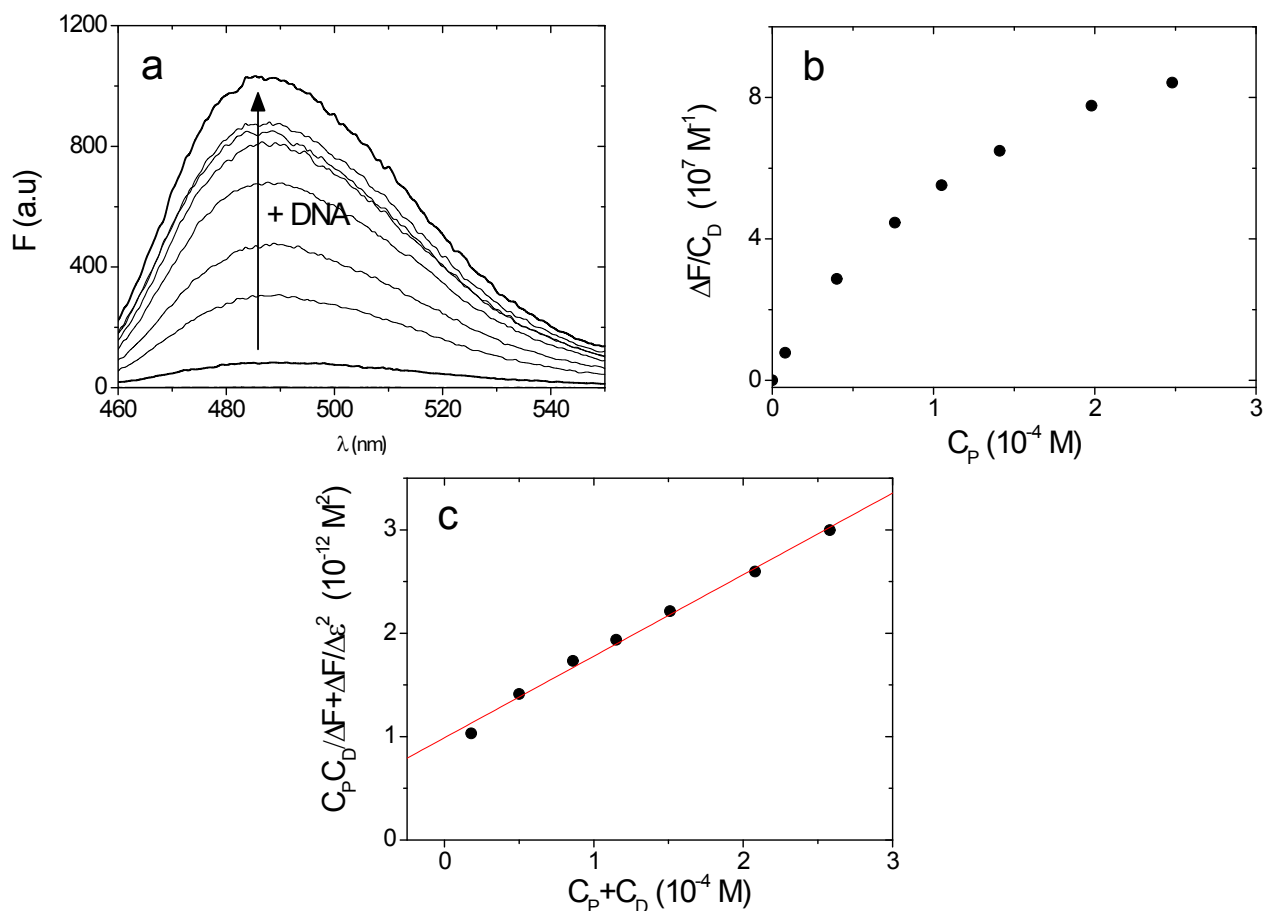
Figure 9S Emission spectra (a, λ_{exc} = 450 nm), binding isotherm (b, λ_{em} = 484 nm) and relevant analysis (c) according to equation (6) for a spectrofluorometric titration of TFT with DNA. C_D = 1.0×10^{-5} M, C_P = 0 to 2.5×10^{-4} M, I = 0.01 M, pH 7.0, T = 25 °C.
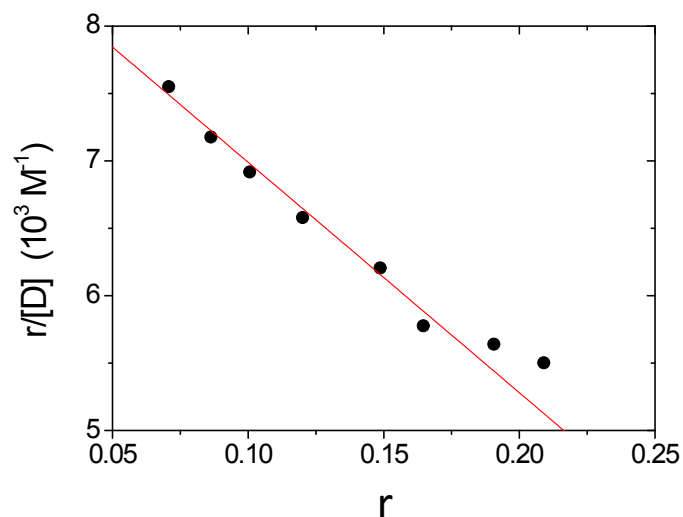
Figure 10S Example of Scatchard plot obtained from absorbance titration data for the TFT/DNA system. $C_D = 5.2 \times 10^{-5}$ M, $C_P = 0$ to 5.1×10^{-4} M, $I = 0.01$ M, pH 7.0, T = 25 °C
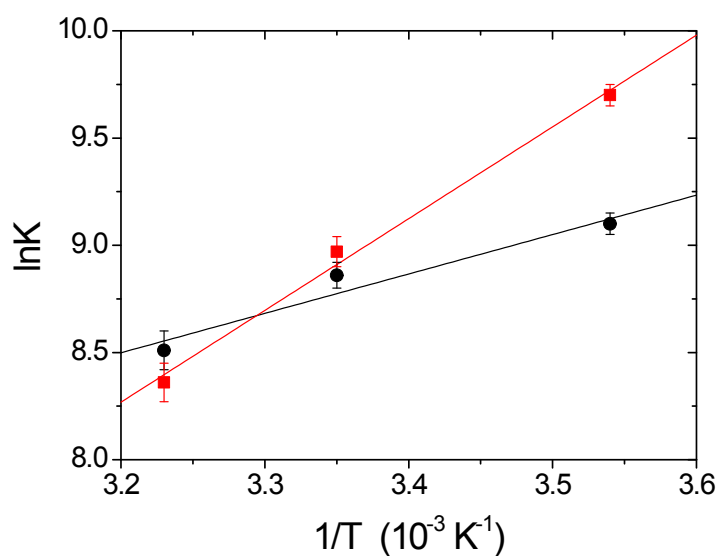
Figure 11S Temperature dependence of the binding constant K for the TFT/DNA system; (●) absorbance, (■) fluorescence, $I = 0.01$ M, pH 7.0, T = 25 °C. The continuous line is drawn according to the equation $\ln K = -\Delta H/RT + \Delta S/R$.
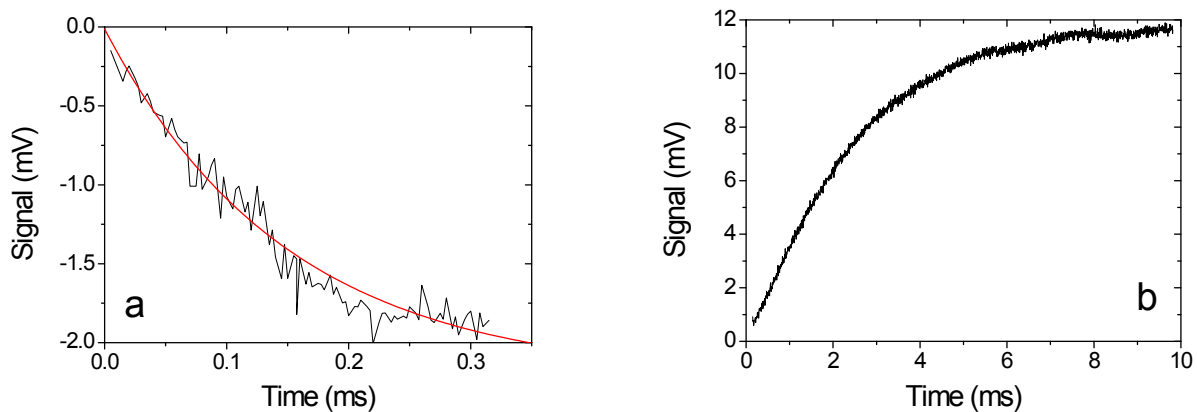
Figure 12S T-jump relaxation curves registered for the TFT/DNA system under low dye and polymer excess conditions; a) fast effect, b) slow effect, $C_D = 2.0 \times 10^{-6}$ M, $C_P = 3.74 \times 10^{-4}$ M. I = 0.11 M, pH 7.0, $\lambda = 450$ nm, T = 25 °C.
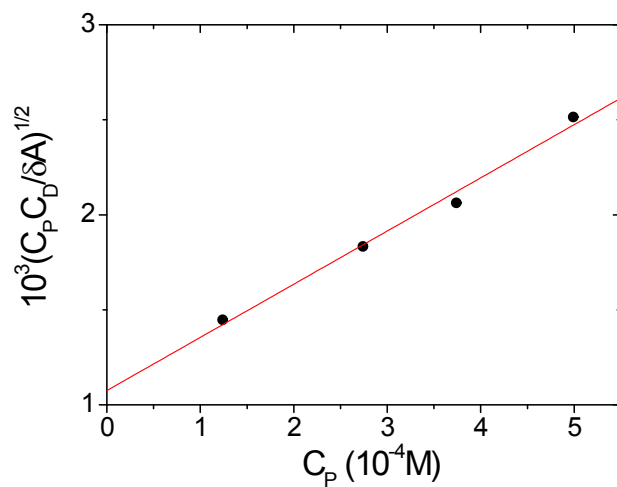
Figure 13S T-jump amplitude analysis for the fast step of the curves registered for the TFT/DNA system under low dye and polymer excess conditions. I = 0.11 M, pH 7.0, T = 25 °C.

Figure 14S T-jump relaxation curves registered for the TFT/DNA system under high dye content conditions; a) overall curve, b) amplification of the fast effect, $C_D = 1.2\times10^{-4}$ M, $C_P = 7.7\times10^{-5}$ M. I = 0.11 M, pH 7.0, $\lambda = 450$ nm, T = 25 °C.

Derivation of equation (4) of the text

Consider reaction scheme (S1.1)-(S1.2)

$$D + D \leftrightharpoons D_2 \tag{S1.1}$$
$$D_2 \leftrightharpoons D_2{}' \tag{S1.2}$$

where D, D_2 and D_2' represent respectively the equilibrium concentration of the dye monomer and dye dimer in two different forms. Let's define also the equilibrium constant of dimerization as
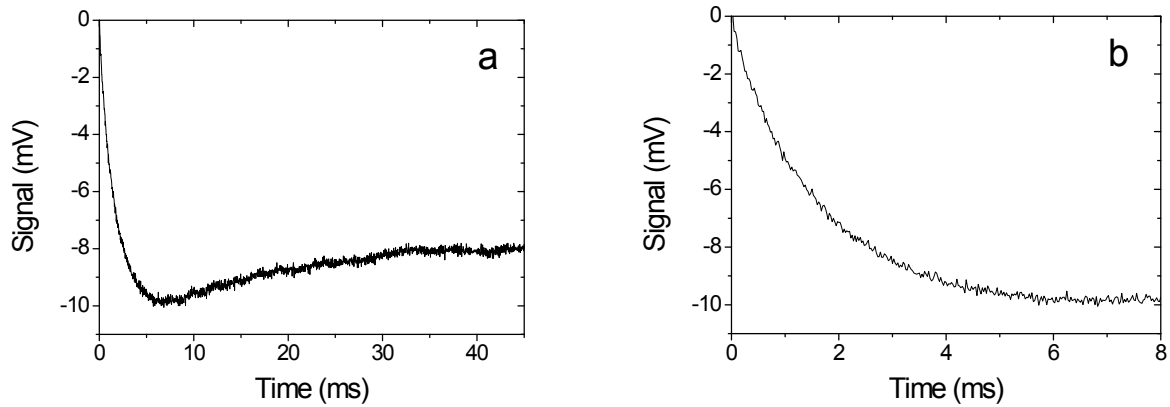
$$K_{dim} = [D_2]/[D]^2 \tag{S1.3}$$

and k_{iso}, k_{-iso} and $K_{iso} = k_{iso}/k_{-iso}$ respectively the forward and backward rate constants and the equilibrium constant of the isomerisation process (S1.2). The mass equation for the dye is

$$C_D = D + 2D_2 + 2D_2' \tag{S1.4}$$

and can be expressed in its differentiated form as follows

$$\delta D + 2\delta D_2 + 2\delta D_2' = 0 \tag{S1.5}$$

where ∂i indicates the deviation from equilibrium of the i-th species caused at the temperature-jump. Differentiation of (S1.3) yields

$$\delta D_2 = 2K_{dim}D\delta D \tag{S1.6}$$

Substitution of (S1.6) in (S1.5) yields equation (S1.7)

$$\frac{\delta D_2}{2K_{dim}D} + 2\delta D_2 + 2\delta D_2' = 0 \tag{S1.7}$$

The differential kinetic law for equation (S1.2) is

$$\frac{d(\delta D_2')}{dt} = k_{iso}\delta D_2 - k_{-iso}\delta D_2' \tag{S1.8}$$

Form equations (S1.8) and (S1.7) we obtain

$$\frac{d(\delta D_2')}{dt} = -\underbrace{\left(\frac{4K_{dim}k_{iso}D}{1 + 4K_{dim}D} + k_{-iso}\right)}_{1/\tau}\delta D_2' \tag{S1.9}$$

whose integration yields the expression for the reciprocal relaxation time $1/\tau$ as (equation (3) of the text)

$$1/\tau = \frac{4K_{dim}k_{iso}D}{1 + 4K_{dim}D} + k_{-iso} \tag{S1.10}$$

The relationship between the monomer concentration D and the total analytical concentration CD can be obtained using equations (S1.3), (S1.4) and the definition of K_{iso}:

$$C_D = D + 2K_{dim}D^2 + 2K_{iso}K_{dim}D^2 \qquad (S1.11)$$

or

$$D^2 + \frac{D}{2K_{dim}(1+K_{iso})} - \frac{C_d}{2K_{dim}(1+K_{iso})} = 0$$
(S1.12)

That is, a second order function with only possible positive solution equal to

$$D = \frac{-\dfrac{1}{2K_{dim}(1+K_{iso})} + \sqrt{\left[\dfrac{1}{2K_{dim}(1+K_{iso})}\right]^2 + \dfrac{2C_d}{K_{dim}(1+K_{iso})}}}{2}$$
(S1.13)

For the analysis of data points according to equation (S1.10) in a first step [D] = C_D approximation is used to obtain first K_{dim} and K_{iso} = k_{iso}/k_{-iso} rough evaluation that enable to calculate [D] and re-plot the data with an iterative procedure, until convergence is reached.

Derivation of equations (11)-(12) of the text

Consider the following reaction scheme

$$\text{(S2.1)}$$

As concerns the fast step, in the fast time scale the slow one can be neglected and the rate law, written in a differential way, is the following

$$-\frac{d\delta D}{dt} = k_f \delta([P] \times [D]) - k_{-f}\delta PD' \qquad (S2.2)$$

As $\delta(P \times D) = P\delta D + D\delta P$ and $\delta D = \delta P = -\delta PD'$ it turns out that

$$-\frac{d\delta D}{dt} = [k_f([P] + [D]) + k_{-f}]\delta D \tag{S2.3}$$

$$-\frac{d\delta D}{\delta D} = [k_f([P] + [D]) + k_{-f}]dt \tag{S2.3}$$

and thus

$$1/\tau_f = k_f([P]+[D]) + k_{-f} \tag{S2.4}$$

Considering now the slow step, the fast one has to be intended as a pre-equilibrium with relevant K_f = k_f/k_{-f} = [PD]/([P]×[D]) constant. The differential mass conservation law respect to the dye is

$$\delta D + \delta PD + \delta PD' = 0 \tag{S2.5}$$

$$\delta D + \delta PD + K_f(P\delta D + D\delta P) = 0 \tag{S2.6}$$

with $\delta D = \delta P$, thus

$$\delta PD = - \{1 + K_f(P + D)\}\, \delta D \tag{S2.7}$$

The rate law, written in a differential way, is the following

$$\frac{d\delta PD}{dt} = k_S\delta([P] \times [D]) - k_{-s}\delta PD \tag{S2.8}$$

$$\frac{d\delta PD}{dt} = -\left\{\frac{k_S([P] + [D])}{1 + K_f([P] + [D])} + k_{-s}\right\}\delta PD \tag{S2.9}$$
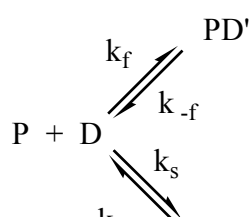
$$\frac{d\delta PD}{\delta PD} = -\left\{\frac{k_S([P] + [D])}{1 + K_f([P] + [D])} + k_{-s}\right\}dt \tag{S2.10}$$

and thus

$$1/\tau_s = k_s([P]+[D])/(1+ K_f([P]+[D])) + k_{-s} \tag{S2.11}$$

Derivation of equation (14) of the text

Consider the following reaction scheme

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$$(S3.1)$$

where the formation of PD (slow) is supposed to be rate determining, whereas the other steps are fast. P and D indicate respectively free polymer and free dye monomer, D_2 is the dye dimer, PD the DNA/dye monomer complex and PD_3 a DNA/dye monomer/dye dimer complex. According to this scheme $K_{dim} = [D_2]/[D]^2$, $K_f = k_f/k_{-f} = [PD']/([P]\times[D])$, $K_s = k_s/k_{-s} = [PD]/([P]\times[D])$ and $K_3 = [PD_3]/([D_2]\times[PD])$.

The mass conservation equations in differential form for polymer and dye are

$$\delta P + \delta PD + \delta PD^{'} + \delta PD_3 = 0 \tag{S3.2}$$

$$\delta D + 2\delta D_2 + \delta PD + \delta PD' + 3\delta PD_3 = 0 \tag{S3.3}$$

Differentiation of the expressions for K_{dim} , K_f and K_3 yield the relationships

$$\delta D_2 = 2K_{dim}D\delta D \tag{S3.4}$$

$$\delta PD^{'} = K_f(P\delta D + D\delta P) \tag{S3.5}$$

$$\delta PD_3 = K_3(D_2\delta PD + PD\delta D_2) = K_3K_{dim}D^2\delta PD + 2K_3K_sK_{dim}(P \times D^2)\delta D \tag{S3.6}$$

Substitution of (S3.4) − (S3.6) in (S3.2) yields

$$\delta P\underbrace{[1 + K_fD]}_{\alpha} + \delta D\underbrace{\left(K_fP + 2K_3K_sK_{dim}P \times D^2\right)}_{\beta} + \delta PD\underbrace{\left(1 + K_3K_{dim}D^2\right)}_{\gamma} = 0 \tag{S3.7}$$

or

$$-\delta P = \frac{\beta}{\alpha}\delta D + \frac{\gamma}{\alpha}\delta PD$$
$$(S3.8)$$

Subtraction of (S3.2) to (S3.3) yields

$$\delta D - \delta P + 2\delta D_2 + 2\delta PD_3 = 0 \tag{S3.9}$$

Substitution of (S3.4) − (S3-8) in (S3.10) yields

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$$\delta D + \frac{\beta}{\alpha}\delta D + \frac{\gamma}{\alpha}\delta PD + 4DK_{dim}\delta D + 2K_3K_{dim}D^2\delta PD + 4K_3K_sK_{dim}(P \times D^2)\delta D = 0$$
(S3.10)

$$\delta D \underbrace{\left[1 + \frac{\beta}{\alpha} + 4DK_{dim} + 4K_3K_sK_{dim}P \times D^2\right]}_{\varepsilon} = -\delta PD \underbrace{\left(\frac{\gamma}{\alpha} + 2K_3K_{dim}D^2\right)}_{\theta}$$
(S3.11)

or

$$\delta PD = -\frac{\varepsilon}{\theta}\delta D$$
(S3.12)

Substituting (S3.12) in (S3.8) one obtains

$$-\delta P = \underbrace{\frac{\left(\frac{\beta}{\alpha} - \frac{\gamma}{\alpha} \times \frac{\varepsilon}{\theta}\right)}{\mu}}_{\mu}\delta D$$
(S3.13)

The rate law, in differential form, is represented by (S3.14)

$$-\frac{d}{dt}(\delta PD' + \delta D + 2\delta D_2) = k_s(P\delta D + D\delta P) - k_{-s}\delta PD$$
(S3.14)

Substitution of (S3.4), (S3.5), (S3.12) and (S3.13) in (S3.14) yields

$$-\frac{d\delta D}{dt} = \left\{\frac{k_s(P - \mu \times D) + k_{-s}\frac{\varepsilon}{\theta}}{K_f(P - \mu \times D) + 1 + 4DK_{dim}}\right\}\delta D$$
(S3.15)

Thus, the expression for $1/\tau'_f$ dependence on the reactant content turns to be

$$1\Big/\tau'_f = \frac{k_s(P - \mu \times D) + k_{-s}\frac{\varepsilon}{\theta}}{K_f(P - \mu \times D) + 1 + 4DK_{dim}}$$
(S3.16)

The equations needed to calculate [D] and [P] are obtained as follows, starting for the mass conservation for dye or polymer respectively.

In particular, for the dye:

$$C_D = D + 2D_2 + PD + 3PD_3$$
(S3.17)

$$C_D = D + 2K_{dim}D^2 + PD + 3K_3PD \cdot D_2 \qquad \text{(S3.18)}$$

$$C_D = D + 2K_{dim}D^2 + K_3P \cdot D + 3K_{dim}K_3K_fPD^3 \qquad \text{(S3.19)}$$

Thus, [D] can be calculated solving the cubic equation (one acceptable value only over the three)

$$D^3 + \frac{2K_{dim}}{3K_{dim}K_3K_fP}D^2 + \frac{1 + K_fP}{3K_{dim}K_3K_fP}D - \frac{C_D}{3K_{dim}K_3K_fP} = 0$$
$$\text{(S3.20)}$$

For the polymer:

$$C_P = P + PD + PD_3 \qquad \text{(S3.21)}$$

$$C_P = P + K_fP \cdot D + K_3PD \cdot D_2 = P + K_fP \cdot D + K_3K_fPK_{dim}D^3$$
$$\text{(S3.22)}$$

$$P = \frac{C_P}{1 + K_fD + K_{dim}K_3K_fD^3}$$
$$\text{(S3.23)}$$

Note that a double iterative procedure is needed to analyse the data. In a first approximation, [P] = C_P, [D] = C_D, [PD] = 0 and K_3 is obtained by data fit to equation (S3.16) (note K_{dim} and K_f are known but K_3 is unknown). In the subsequent step [P], [D] and [PD] = K_f[P][D] are calculated from K_f, K_{dim} and K_3 approximate value. Note that the calculation of [D] requires also [P] to be known (and vice versa, equations S3.20 and S3.23). Thus, in a first approximation [D] values are obtained for [P] = C_P and used in equation (S3.23) to get [P] and re-insert these values in equation (S3.23) and so on, until convergence is reached. The converged [P], [D] and [PD] values are used to obtain a new K_3 from equation (S3.16) and so on, until convergence is reached.

## Derivation of the relationship between relaxation curve amplitude and dye content

Consider reaction scheme (S4.1)-(S4.2)

$$D + D \rightleftharpoons D_2 \qquad \text{(S4.1)}$$
$$D_2 \rightleftharpoons D_2' \qquad \text{(S4.2)}$$

where D, D_2 and D_2' represent respectively the equilibrium concentration of the dye monomer and dye dimer in two different forms. These two equations are coupled together.

Since the two reactions are thermodynamically independent, in principle two different kinetic effects could be observed, whose amplitude can be expressed on the basis of normal reactions. If the

coefficients that multiply the elementary reactions (S4.1) and (S4.2) equations in order to find the two normal modes of reaction are respectively 1 and γ_k, the two normal reactions are described by eq.(S4.3), which results from adding γ_k times reaction (S4.2) to reaction (S4.1) (M. Citi et al., 1988)

$$D + D \;\leftrightarrows\; (1 - \gamma_k)\, D_2 + \gamma_k\, D_2' \qquad\qquad k = 1,2 \tag{S4.3}$$
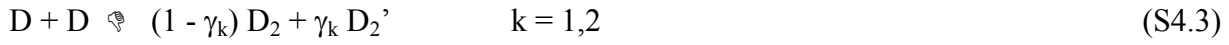
The γ_K values are obtained by solving the following system

$$\begin{vmatrix} r_1 g_{11} - \lambda_k & r_1 g_{12} \\ r_2 g_{21} & r_2 g_{22} - \lambda_k \end{vmatrix} \begin{Vmatrix} 1 \\ \gamma_k \end{Vmatrix} = 0 \tag{S4-4}$$

that means to solve equations as
$$(r_1 g_{11} - \lambda_k) \times 1 + r_1 g_{12} \gamma_k = 0 \tag{S4-5}$$

Eq. (S4-5) yields
$$\gamma_k = \frac{-\left(r_1 g_{11} - \lambda_k\right)}{r_1 g_{12}} \tag{S4-6}$$

where $g_{ij} = g_{ji}$ and the g_{ij} coefficients are related to the equilibrium concentrations by the equations (G. W. Castellan, 1963)

$$g_{11} = \frac{4}{[D]} + \frac{1}{[D_2]} \tag{S4-7}$$

$$g_{12} = -\frac{1}{[D_2]} \tag{S4.8}$$

$$g_{22} = \frac{1}{[D_2]} + \frac{1}{[D_2']} \tag{S4.9}$$

and r_k are the exchange rate of the reactions (S4.1) and (S4.2).

$$r_1 = k_1 [D]^2 = k_{-1} [D_2] \tag{S4.10}$$

$$r_2 = k_2 [D_2] = k_{-2} [D_2'] \tag{S4.11}$$

For $k = 1$, $\lambda_k = \lambda_1$. For $\lambda_1 \gg \lambda_2$ (i.e. (S4.1) much faster than (S4.2), as supposed) it turns out that $\lambda_1 = r_1 g_{11}$, hence $\gamma_1 = 0$ and eq. (IV.24) becomes

$$D + D \;\leftrightarrows\; D_2 \tag{S4.12}$$

Hence, the first normal reaction coincides with the elementary reaction (S4.1).

For $k = 2$, $\lambda_k = \lambda_2$ and, being $\lambda_2 \ll \lambda_1 = r_1 g_{11}$ we have

$$\gamma_2 = -\frac{g_{11}}{g_{12}} = -\frac{4/[D] + 1/[D_2]}{1/[D_2]} = -(1 + 4K_{dim}[D]) \tag{S4.13}$$

with $K_{dim} = [D_2]/[D]^2$ equilibrium constant of dimerization. Thus, the second normal reaction will be

$$2D - (2+4K_{dim}D) \, D_2 \leftrightharpoons -(1+4KdimD)D_2' \tag{S4.14}$$

Now, for a signal recorded with absorbance detection

$$\Delta Abs = \Gamma \Delta\varepsilon \frac{\Delta H}{RT^2} dT = \Gamma \times const \tag{S4.15}$$

and the expression for Γ can be obtained from the normal reaction (S4.14)

$$\Gamma^{-1} = \frac{(2)^2}{[D]} + \frac{(2+4K_{dim}[D])^2}{[D_2]} + \frac{(1+4K_{dim}[D])^2}{[D_2']} \tag{S4.16}$$

Thus, finally

$$\Delta Abs = \frac{const}{\dfrac{(2)^2}{[D]} + \dfrac{(2+4K_{dim}[D])^2}{[D_2]} + \dfrac{(1+4K_{dim}[D])^2}{[D_2']}} \tag{S4.17}$$

The evaluation of Γ^{-1} can be done as K_{dim} is known from the analysis of the relaxation times and [D], [D$_2$] and [D$_2$'] can be calculated from K_{dim} and K_{iso} (Table 1 of the text). The values obtained excellently reproduce the experimental trend of ΔAbs (const = 2.86×10^4 M^{-1}).