Supporting Information

Fourier Transform to Analyze Reaction–Diffusion Dynamics in a Microsystem

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• Theoretical derivation of the expression of the Fourier transform of the concentrations \( R(x,y) \) and \( P(x,y) \) with respect to \( y \);

• Extraction of the dynamic parameters from the stationary reaction-migration-diffusion pattern of the fluorescence signal;

• Independent extraction, by fluorescence spectroscopy, of the thermodynamic constant \( K \) and rate constants \( k_1 \) and \( k_2 \).

• Independent evaluation of the diffusion coefficients, \( d_R \) and \( d_P \), and of the velocities, \( v_R \) and \( v_P \), of the oligonucleotides;

• Experimental constraints to retrieve the kinetic information from the reaction-migration-diffusion pattern.
Theoretical derivation of the expression of the Fourier transform of the concentrations \( R(x, y) \) and \( P(x, y) \) with respect to \( y \)

We consider that the reactive system contains three species, \( \mathbf{R} \), \( \mathcal{R} \), and \( \mathbf{P} \), which are involved in the chemical reaction:

\[
\mathbf{R} + \mathcal{R} \quad \xrightarrow{k_1} \quad \mathbf{P} \quad \xrightarrow{k_2}
\]

where \( k_1 \) and \( k_2 \) designate the rate constants associated to the forward and backward reactions, respectively. We suppose that the reactant \( \mathcal{R} \) is in great excess with respect to \( \mathbf{R} \) and \( \mathbf{P} \) (\( \mathcal{R} \gg R \) and \( \mathcal{R} \gg P \)) and has a constant uniform concentration. We correspondingly introduce \( k_1 \mathcal{R} \) as an effective forward rate constant. Under such conditions, reaction (1) reduces to the simple two-state exchange process between \( \mathbf{R} \) and \( \mathbf{P} \)

\[
\mathbf{R} \quad \xrightarrow{k_1 \mathcal{R}} \quad \mathbf{P} \quad \xrightarrow{k_2}
\]

which apparent thermodynamic constant is \( K' = \frac{k_1 \mathcal{R}}{k_2} \).

To calculate the stationary reaction-migration-diffusion pattern described in the Main Text, we assume that the \( \mathbf{R} \) and \( \mathbf{P} \) motion occurs in a two-dimensional (2D) medium defined by \( 0 \leq x \leq L, -\infty < y < +\infty \). This medium is submitted to a uniform constant electric field \( \overrightarrow{\mathbf{E}} = E\overrightarrow{u_x} \), where \( \overrightarrow{u_x} \) is the unit vector along \( x \). The diffusion coefficients of species \( \mathbf{R} \) and \( \mathbf{P} \) are respectively denoted \( d_R \) and \( d_P \) and their velocities (along the \( x \)-axis) \( v_R \) and \( v_P \). \( \overrightarrow{\mathbf{E}} \) is chosen to impose the migration of the averaged species \( \{\mathbf{R}, \mathbf{P}\} \) in the direction of increasing \( x \).Considering at the \((x=0, y=0)\) origin a \( \{\mathbf{R}, \mathbf{P}\} \) source associated to the equilibrium conditions \( R(0,0) \) and \( P(0,0) \), we look for stationary concentrations profiles, \( R(x,y) \) and \( P(x,y) \), obeying the following partial differential equations:

\[
-v_R \frac{\partial R(x,y)}{\partial x} + d_R \left[ \frac{\partial^2 R(x,y)}{\partial x^2} + \frac{\partial^2 R(x,y)}{\partial y^2} \right] - k_1 \mathcal{R} R(x,y) + k_2 P(x,y) = 0 \quad (3)
\]

\[
-v_P \frac{\partial P(x,y)}{\partial x} + d_P \left[ \frac{\partial^2 P(x,y)}{\partial x^2} + \frac{\partial^2 P(x,y)}{\partial y^2} \right] + k_1 \mathcal{R} R(x,y) - k_2 P(x,y) = 0 \quad (4)
\]
After Fourier transform along $y$, Eqs. (3,4) lead to

$$\frac{\partial^2 \tilde{R}(x,q)}{\partial x^2} - \frac{v_R}{d_R} \frac{\partial \tilde{R}(x,q)}{\partial x} - \left( q^2 + \frac{k_1 R}{d_R} \right) \tilde{R}(x,q) = -\frac{k_2}{d_R} \tilde{P}(x,q)$$  \hspace{-1cm} (5)

$$\frac{\partial^2 \tilde{P}(x,q)}{\partial x^2} - \frac{v_P}{d_P} \frac{\partial \tilde{P}(x,q)}{\partial x} - \left( q^2 + \frac{k_2}{d_P} \right) \tilde{P}(x,q) = -\frac{k_1 R}{d_P} \tilde{R}(x,q)$$  \hspace{-1cm} (6)

where $\tilde{R}(x,q) = 1/\sqrt{2\pi} \int_{-\infty}^{\infty} R(x,y)e^{-iqy}dy$ and $\tilde{P}(x,q) = 1/\sqrt{2\pi} \int_{-\infty}^{\infty} P(x,y)e^{-iqy}dy$.

We restrict our analysis to a regime where diffusion along $x$ is negligible with respect to migration:  \footnote{The following relations correspond to the conditions: $\frac{L_x}{\delta_y} \gg 1$ and $\frac{L_x}{\delta_P} \gg 1$ where $L_x$ designate the image length along the $x$-axis. Such conditions were always fulfilled in the present study.}

$$\frac{\partial^2 \tilde{R}}{\partial x^2} \ll \frac{v_R}{d_R} \frac{\partial \tilde{R}}{\partial x}$$ \hspace{-1cm} (7)

$$\frac{\partial^2 \tilde{P}}{\partial x^2} \ll \frac{v_P}{d_P} \frac{\partial \tilde{P}}{\partial x}$$ \hspace{-1cm} (8)

Thus, Eqs.(5,6) become:

$$\frac{\partial \tilde{R}(x,q)}{\partial x} = -\frac{1}{v_R} \left( d_R q^2 + k_1 R \right) \tilde{R}(x,q) + \frac{1}{v_R} k_2 \tilde{P}(x,q)$$ \hspace{-1cm} (9)

$$\frac{\partial \tilde{P}(x,q)}{\partial x} = \frac{1}{v_P} k_1 R \tilde{R}(x,q) - \frac{1}{v_P} \left( d_P q^2 + k_2 \right) \tilde{P}(x,q)$$ \hspace{-1cm} (10)

Eventually, we introduce the variables $\kappa_1 = \frac{k_1 R}{v_R}$, $\kappa'_1 = \kappa_1 \frac{v_P}{v_R}$, $\kappa_2 = \frac{k_2}{v_R}$, $\kappa'_2 = \kappa_2 \frac{v_P}{v_R}$, $\delta_R = \frac{d_R}{v_R}$, and $\delta_P = \frac{d_P}{v_P}$ to simplify the Eqs.(9,10) expressions:

$$\frac{\partial \tilde{R}(x,q)}{\partial x} = -\left( \delta_R q^2 + \kappa_1 \right) \tilde{R}(x,q) + \kappa'_2 \tilde{P}(x,q)$$ \hspace{-1cm} (11)

$$\frac{\partial \tilde{P}(x,q)}{\partial x} = \kappa'_1 \tilde{R}(x,q) - \left( \delta_P q^2 + \kappa_2 \right) \tilde{P}(x,q)$$ \hspace{-1cm} (12)

The latter linear system admits solutions in the form:

$$\tilde{R}(x,q) = R_+ e^{\lambda_+ x} + R_- e^{\lambda_- x}$$ \hspace{-1cm} (13)

$$\tilde{P}(x,q) = P_+ e^{\lambda_+ x} + P_- e^{\lambda_- x}$$ \hspace{-1cm} (14)

where the eigenvalues obey:

$$\lambda_{\pm} = -\frac{1}{2} \left[ (\kappa_1 + \kappa_2) + (\delta_R + \delta_P) q^2 \right] \pm \frac{1}{2} \sqrt{\left[ (\kappa_1 + \kappa_2) + (\delta_R - \delta_P) q^2 \right]^2 + 4(\delta_R - \delta_P) q^2 \kappa_2}$$ \hspace{-1cm} (15)
and where the amplitudes are given by:

\[ R_+ = \frac{1}{(\lambda_+ - \lambda_-)} \left[ -(\delta Rq^2 + \kappa_1 + \lambda_-) \hat{R}(0,q) + \kappa_2 \hat{P}(0,q) \right] \]  

(16)

\[ R_- = \frac{1}{(\lambda_+ - \lambda_-)} \left[ (\delta Rq^2 + \kappa_1 + \lambda_+) \hat{R}(0,q) - \kappa_2 \hat{P}(0,q) \right] \]  

(17)

\[ P_+ = \frac{1}{(\lambda_+ - \lambda_-)} \left[ \kappa'_1 \hat{R}(0,q) - (\delta pq^2 + \kappa_2 + \lambda_-) \hat{P}(0,q) \right] \]  

(18)

\[ P_- = \frac{1}{(\lambda_+ - \lambda_-)} \left[ -\kappa'_1 \hat{R}(0,q) + (\delta pq^2 + \kappa_2 + \lambda_+) \hat{P}(0,q) \right] \]  

(19)

\( \hat{R}(0,q) \) and \( \hat{P}(0,q) \) are here the Fourier transforms of the \( R(0,y) \) and \( P(0,y) \) initial conditions, respectively.

Finally, Eqs. (16–19) can be transformed according to the following. First, we introduce \( \tilde{G}(0,q) \), a \( q \)-dependent function which reflects the geometry of the channel at the entry of the measurement chamber:

\[ \tilde{G}(0,q) = \frac{\hat{R}(0,q)}{\hat{R}(0,0)} = \frac{\hat{P}(0,q)}{\hat{P}(0,0)}. \]

By doing so, we assume that the normalized Fourier transform of the concentration profile at \( x = 0 \) is independent on the solute nature, which could be verified experimentally.\(^2\) Second, we make use of the equilibrium condition before injection, \( K' = P(0,0)/R(0,0) \). Eqs. (16–19) become Eqs. (20–23):

\[ R_+ = - \frac{1}{(\lambda_+ - \lambda_-)} (\delta Rq^2 + \lambda_-) \tilde{G}(0,q) \frac{1}{1 + K'} \frac{C_0}{1 + K'} \]  

(20)

\[ R_- = \frac{1}{(\lambda_+ - \lambda_-)} (\delta Rq^2 + \lambda_+) \tilde{G}(0,q) \frac{1}{1 + K'} \frac{C_0}{1 + K'} \]  

(21)

\[ P_+ = - \frac{1}{(\lambda_+ - \lambda_-)} (\delta pq^2 + \lambda_-) \tilde{G}(0,q) \frac{K'}{1 + K'} \frac{C_0}{1 + K'} \]  

(22)

\[ P_- = \frac{1}{(\lambda_+ - \lambda_-)} (\delta pq^2 + \lambda_+) \tilde{G}(0,q) \frac{K'}{1 + K'} \frac{C_0}{1 + K'} \]  

(23)

where \( C_0 = R(0,0) + P(0,0) \) designates the total concentration in \( R \) and \( P \) in the loaded sample.

\(^2\)In the present microsystem, the initial condition is not taken at the entry of the measurement chamber but at the abscissa where the electric field becomes constant.\(^1\) Thus \( \tilde{G}(0,q) \) reflects not only the shape of the channel, but also some broadening by migration, due to out-of-axis electric fields between the injection nozzle and the zone of homogeneous velocity, as well as by molecular diffusion. We demonstrated in a previous report that the width of the corresponding initial condition was gaussian with a typical 60 \( \mu \text{m} \) half-width that did not depend on the solute, whatever the investigated sample (oligonucleotide or dsDNA).\(^1\) In the case where the geometry of the initial profiles of \( R \) and \( P \) would not be identical, it would be necessary to keep the \( R(0,q) \) and \( P(0,q) \) terms in the amplitudes expressions of the exponential terms.
Extraction of the dynamic parameters from the stationary reaction-migration-diffusion pattern of the fluorescence signal

Theoretical expression of the stationary reaction-migration-diffusion pattern of the fluorescence signal

Introducing \( Q_R \) and \( Q_P \) as, respectively, the \( R \) and \( P \) brightness, the theoretical \( q \)-dependence of \( \hat{F}(x,q) \), the stationary reaction-migration-diffusion pattern observed by fluorescence video microscopy, can be derived from Eqs.(13–14) and Eqs.(20–23):

\[
\hat{F}(x,q) = \mathcal{F}_+ e^{\lambda_+ x} + \mathcal{F}_- e^{\lambda_- x} \tag{24}
\]

with the decays \( \lambda_+ \) and \( \lambda_- \) given by Eqs.(15) and the amplitudes \( \mathcal{F}_+ \) and \( \mathcal{F}_- \) by:

\[
\mathcal{F}_+ = -\frac{1}{(\lambda_+ - \lambda_-)} \left[ (\delta_Rq^2 + \lambda_-)Q_R \frac{1}{1 + K'} + (\delta_Pq^2 + \lambda_+)Q_P \frac{K'}{1 + K'} \right] \hat{G}(0,q)C_0 \tag{25}
\]

\[
\mathcal{F}_- = \frac{1}{(\lambda_+ - \lambda_-)} \left[ (\delta_Rq^2 + \lambda_+)Q_R \frac{1}{1 + K'} + (\delta_Pq^2 + \lambda_-)Q_P \frac{K'}{1 + K'} \right] \hat{G}(0,q)C_0 \tag{26}
\]

In practice, to correct from any illumination homogeneity or photobleaching, the \( \hat{F}(x,q) \) pattern is normalized by the zeroth mode \( \hat{F}(x,0) \) which is equal to \((Q_R \frac{1}{1 + K'} + Q_P \frac{K'}{1 + K'})C_0 \) (see Eqs. (15) and (24–26)). We thus have:

\[
\frac{\hat{F}(x,q)}{\hat{F}(x,0)} = \mathcal{F}_+^{\text{norm}} e^{\lambda_+ x} + \mathcal{F}_-^{\text{norm}} e^{\lambda_- x} \tag{27}
\]

with

\[
\mathcal{F}_+^{\text{norm}} = -\frac{1}{(\lambda_+ - \lambda_-)} \left[ (\delta_Rq^2 + \lambda_-)I_R + (\delta_Pq^2 + \lambda_+)I_P \right] \hat{G}(0,q) \tag{28}
\]

\[
\mathcal{F}_-^{\text{norm}} = 1 - \mathcal{F}_+^{\text{norm}} \tag{29}
\]

We introduced here the respective \( R \) and \( P \) fractionary fluorescence intensity:

\[
I_R = \frac{1}{1 + QK'} \tag{30}
\]

\[
I_P = \frac{QK'}{1 + QK'} \tag{31}
\]

and the relative brightness of \( P \) with regard to \( R \) : \( Q = \frac{Q_P}{Q_R} \).
**Extraction of the dynamic parameters**

Seven parameters characterize the association dynamics between $R$ and $\mathcal{R}$: $d_R$, $d_P$, $v_R$, $v_P$, $k_1 R$, $k_2$, and $Q$. We assume that $d_R$ and $v_R$ have first been measured with the same microsystem during a preliminary experiment performed by injecting $R$ only.\cite{1} Thus five parameters, $d_P$, $v_P$, $k_1 R$, $k_2$, and $Q$, remain to be determined from analyzing the stationary reaction-migration-diffusion pattern of the fluorescence signal. As shown in the following, the four first ones can be extracted from the spatial analysis of the $\hat{F}(x,q)$ collection whereas $Q$ can be obtained from the corresponding amplitude analysis. From an experimental point of view, analyzing the decay rates is particularly advantageous since the spatial dependence of $\hat{F}(x,q)$ does not depend on the shapes of the initial concentration profiles $\hat{R}(0,q)$ and $\hat{P}(0,q)$ (contained in the $\hat{G}(0,q)$ function).

The spatial analysis of the collection of the $\hat{F}(x,q)$ terms provides the $q$-dependence of the eigenvalues $\lambda_+$ and $\lambda_-$\footnote{From an experimental point of view, it is essential to collect a large set of $\lambda_+(q)$ and $\lambda_-(q)$ to reliably extract $k_1$, $k_2$, $\delta_R$, and $\delta_P$. In particular, it is important to reach an asymptotic regime dominated by diffusion only (vide supra). It can either be the one of “slow” or “fast” chemistry. In the investigated experimental system, we decreased the applied electric field to be able to reliably measure $\lambda_+$ and $\lambda_-$ for the smallest $q$ values associated to a regime of fast exchange between $R$ and $P$.} from which $k_1$, $k_2$, and $\delta_P$ can be extracted ($\delta_R$ has been measured in a preliminary experiment)\footnote{This conclusion can be evidenced by considering the $q$-dependence of $\lambda_+ + \lambda_- = -(k_1 + k_2) - (\delta_R + \delta_P)q^2$ and of $\lambda^2_+ - \lambda^2_- = (\delta_R + \delta_P)(\delta_R - \delta_P)q^2$ easily derived from Eqs.(15).}. Data are subsequently analyzed in the following way:

- Beyond the relaxation time $\tau_x = \frac{1}{k_1 R_k + k_2}$, the reactive system submitted to the reaction (1) behaves as if it would contain one averaged species $\{R,P\}$ only. At low enough applied voltage $u$, the velocity along the $x$-axis of the latter, $v^x_{[R,P]} = \frac{1}{1+K'} v^R_R + \frac{K'}{1+K'} v^p_P$, can be independently measured by recording beyond $x = v^x_{[R,P]} \tau_x$ the introduction of the fluorescent front of the mixture into the analysis chamber. Then the velocity $v_{[R,P]} = \frac{1}{1+K'} v^R_R + \frac{K'}{1+K'} v^p_P$ is deduced from the actual voltage $U$ during the experiment as $v_{[R,P]} = \frac{U}{u} v^y_{[R,P]}$ (See Figure S-6 in reference [1]);

- As expressed above, $v_{[R,P]}$ depends on $v_R$, $v_P$, and $K'$. The ratio $\frac{v^y_{[R,P]}}{v^x_{[R,P]}}$ provides a second independent observable linking $v_R$, $v_P$, and $K'$. $v_R$ being known

\footnote{$K'$ can be calculated from $k_1$ and $k_2$.}
(vide supra), $v_P$ and $K'$ can be obtained;

- $k_1$ is derived from $\kappa_1$ by using the value of $v_R$. Then $k_2$, is extracted from the values of $K'$ and $k_1\mathcal{R}$;

- $d_P$ is eventually obtained from $\delta_P$ and $v_P$.

Once $d_R$, $d_P$, $v_R$, $v_P$, $k_1\mathcal{R}$, and $k_2$ have been obtained, $Q$ can be extracted from the ratio $\frac{\mathcal{F}_{+\text{norm}}(q)}{\mathcal{F}_{-\text{norm}}(q)}$, i.e., the amplitude analysis of the collection of the $\tilde{\mathcal{F}}(t,q)$ terms.
Independent extraction, by fluorescence spectroscopy, of the thermodynamic constant $K$ and rate constants $k_1$ and $k_2$

Fluorescence measurements were performed on a LPS 220 spectrofluorimeter (Photon Technology International, Birmingham, NJ) in 25/50 mM NaOH/Hepes, 1.25/5 mM Mg(OH)$_2$/Hepes buffer, pH 7.5, supplemented with 10 µg/mL sonicated DNA salmon sperm, to prevent oligonucleotide adsorption, and 0.1% (w/w) PDMA (T = 20°C). A quartz cuvette with a 1 cm optical path was used; excitation and emission wavelengths were respectively set at 587 and 612 nm with a bandwidth of 4 nm. After each experiment, the cuvette was cleaned for 15 minutes with a 1% (v/v) Hellmanex soap (Hellma, Mulheim, Germany) in an ultrasonic bath.

**Thermodynamic measurements**

400 µL of 50 nM tex-9 were equilibrated at 20°C. The decrease in fluorescence intensity, associated to the hybridization process, was recorded while increasing concentrations of M100 were added at constant tex-9 concentration (Figure 1S). Data were then analyzed on the basis of reaction (1), for which the following formula can be easily obtained:

$$\frac{I_{eq}}{I_0} = 1 - \frac{1}{2}(1 - Q) \left\{ \left( 1 + \xi + \frac{1}{KR_0} \right) - \sqrt{\left( 1 + \xi + \frac{1}{KR_0} \right)^2 - 4\xi} \right\}, \quad (32)$$

where $I_{eq}$ is the fluorescence intensity at equilibrium when $\xi = \frac{R}{R_0}$ equivalents of species $R$ (ie M100) have been added, $I_0$ the intensity at the beginning of the experiment, $R_0$ the initial concentration in tex-9, and $Q$ the relative brightness of the duplex with regards to the free 9mer. $R_0$ being known, we extract $K$ and $Q$ by a least-squares fit. Results are displayed in Figure 1S.
**Figure 1S.** Normalized fluorescence intensity at equilibrium vs. the number of equivalents of M100 added to a 50 nM tex-9 solution. The solid line corresponds to the fit according to Eq. (32). We extracted $K = 3.4 \pm 0.6 \times 10^5$ and $Q = 0.75 \pm 0.02$.

**Kinetic measurements**

Using a RX2000 rapid kinetic stopped flow accessory (Applied Photophysics, Leatherhead, UK), two 200 µL solutions, A and B, were mixed with typical dead times of 100 ms and the fluorescence intensity was recorded over time at either 10 or 100 Hz. In association experiments, solution A contained tex-9 and solution B M100 (final concentrations 50 nM and 10 µM, respectively). In dissociation experiments, solution A contained an equilibrated mixture of tex-9+M100 (final concentrations 50 nM and 10 µM, respectively) and solution B an unlabeled oligonucleotide having a much stronger affinity for M100 than tex-9 (U13 – final concentration 50 µM).

**Model for association experiments**

The association reaction (1) reduces to (2) when $R$ is in excess with respect to $R$. The kinetic law associated to reaction (2) in a homogeneous solution is

$$\frac{dR(t)}{dt} = -k_1 R(t) + k_2 P(t).$$

(33)

Considering the initial condition $R_0$ and the conservation of matter, $R(t) + P(t) = R_0$, Eq. (33) yields the solution:

$$R(t) = R_{eq} + [R_0 - R_{eq}] e^{-t/\tau},$$

(34)
where $R_{eq}$ is the concentration of $R$ at equilibrium and $\tau = (k_1 R + k_2)^{-1}$. Eq.(34) can be eventually used to derive the temporal dependence of the normalized fluorescence intensity $I(t)/I_0$:

$$\frac{I(t)}{I_0} = \frac{I_{eq}}{I_0} + \left(1 - \frac{I_{eq}}{I_0}\right) e^{-t/\tau} \tag{35}$$

where

$$\frac{I_{eq}}{I_0} = \frac{1 + K'Q}{1 + K''} \tag{36}$$

**Model for dissociation experiments**

To determine $k_2$, we performed displacement experiments in which the unlabeled oligonucleotide U13 ($R_{NF}$) hybridized with M100 ($R$) and took the place of tex-9 ($R$) in the fluorescent duplex $P$ to form a much more stable non-fluorescent duplex ($P_{NF}$). The corresponding kinetic scheme can be written:

$$\begin{align*}
P & \xrightarrow{k_2} R + R \\
& \xrightarrow{k_1} R_{NF} + R & \Rightarrow & P_{NF} \tag{37} \end{align*}$$

When the concentrations are chosen to make the dissociation of the fluorescent duplex $P$ rate-limiting with regards to the subsequent formation of non-fluorescent duplex $P_{NF}$, the time dependence of the $R$ concentration is given by:

$$R(t) = R_{eq} + (R_0 - R_{eq}) e^{-k_2 t} \tag{39}$$

where $R_0$ and $R_{eq}$ now designate the initial and final equilibrium concentrations of species $R$ during dissociation experiments. The corresponding temporal evolution of the normalized fluorescence intensity $I(t)/I_0$ is:

$$\frac{I(t)}{I_0} = \frac{I_{eq}}{I_0} + \left(1 - \frac{I_{eq}}{I_0}\right) e^{-k_2 t} \tag{40}$$

where

$$\frac{I_{eq}}{I_0} = \frac{R_{eq} + QP_{eq}}{R_0 + QP_0} \tag{41}$$
Analysis of the experimental results

The results of the association and dissociation experiments are displayed in Figure 2Sa and 2Sb, respectively. \( k_2 \) is first extracted from fitting the experimental data in Figure 2Sb. \( k_1 \mathcal{R} \) (or similarly \( k_1 \)) is subsequently derived using the \( \tau \) value extracted from the temporal dependence displayed in Figure 2Sa. Eventually, the relative brightness \( Q \) is obtained from analyzing the amplitude of the temporal dependence in Figure 2Sa, introducing the value of \( K = \frac{k_1}{k_2} \) in Eq.(36).

![Figure 2Sa and 2Sb](image)

**Figure 2S.** Normalized fluorescence intensity for the association (a) and dissociation (b) of 50 nM tex-9 + 10 \( \mu M \) M100. Solid lines correspond to monoexponential fits according to Eqs (35) and (40) respectively. We extracted \( k_1 \mathcal{R} = 1.9 \pm 0.1 \text{ s}^{-1} \) (\( k_1 = 1.9 \pm 0.1 \text{ M}^{-1} \text{s}^{-1} \)), \( k_2 = 0.42 \pm 0.01 \text{ s}^{-1} \) (\( \tau = 2.4 \pm 0.1 \text{ s}^{-1} \)), and \( Q = 0.76 \).
Independent evaluation of the diffusion coefficients, $d_R$ and $d_P$, and of the velocities, $v_R$ and $v_P$, of the oligonucleotides

The diffusion coefficient and the velocity of tex-9 (R) can directly be measured as described in reference [1], using the protocol established for a pure species. The experiment was performed at 20°C in 25/50 mM NaOH/Hepes, 1.25/5 mM Mg(OH)$_2$/Hepes buffer, pH 7.5. A 1 μM oligonucleotide sample was injected in the analysis chamber upon applying a 300 V voltage drop. We extracted a diffusion coefficient and a mobility equal to 153 ± 10 μm$^2$s$^{-1}$ and $20 \pm 2 \times 10^{-9}$ m$^2$V$^{-1}$s$^{-1}$, respectively.

In contrast, the diffusion coefficient and the velocity of the duplex (P), resulting from the tex-9 hybridization with M100 (R), cannot be directly obtained at 20°C and at micromolar concentrations using the reference [1] protocol established for a non-reactive binary mixture. Indeed kinetics is precisely present under such experimental conditions. Alternatively, we relied on measurements performed on the duplex formed by tex-9 hybridization with C100, the M100 analog bearing the perfect matched sequence. The tex-9/C100 duplex is more stable than the tex-9/M100 one and it does not dissociate at the timescale of the experiment; consequently, we can now apply the protocol reported in reference [1] for a binary mixture to extracted the parameters sought for. Note that mispairing in tex-9/M100 is not expected to introduce any significant change of diffusion coefficient or velocity with regards to results that could be obtained on tex-9/C100. We injected at 20°C a mixture of tex-9 and C100, 1 and 10 μM respectively, and applied the binary mixture biexponential fitting procedure while setting the tex-9 diffusion coefficient at 153 ± 10 μm$^2$s$^{-1}$. From this experiment, the P diffusion coefficient and mobility were respectively evaluated to 40 ± 4 μm$^2$s$^{-1}$ and $20 \pm 2 \times 10^{-9}$ m$^2$V$^{-1}$s$^{-1}$. 
Experimental constraints to retrieve the kinetic information from the reaction-migration-diffusion pattern

The expression of the eigenvalues given in Eqs.(15) can be much simplified according to the relative values of two apparent relaxation rates: \((\kappa_1 + \kappa_2)\) that characterize the species reactivity and \((\delta_R - \delta_P)q^2\) that governs the diffusive behavior difference between \(R\) and \(P\) at the spatial scale \(q\).

- If \((\kappa_1 + \kappa_2) \ll (\delta_R - \delta_P)q^2\), diffusion dominates chemistry for relaxation towards equilibrium at the spatial scale \(q\). The mixture seems non reactive; its components \(R\) and \(P\) diffuse independently with apparent diffusion coefficients \(\delta_R\) and \(\delta_P\), respectively. Introducing \(K' = \frac{\delta_P}{\delta_R}\), the apparent thermodynamic constant associated to the reaction (2), we have \(\hat{R}(x,q) \simeq \hat{G}(0,q)\frac{1}{1+K'}C_0\exp(-\delta_R q^2 x)\) and \(\hat{P}(x,q) \simeq \hat{G}(0,q)\frac{K'}{1+K'}C_0\exp(-\delta_P q^2 x)\) at zeroth order;

- If \((\kappa_1 + \kappa_2) \gg (\delta_R - \delta_P)q^2\), chemistry dominates diffusion for relaxation towards equilibrium at the spatial scale \(q\): The reactive mixture behaves as a single component with a \(\frac{1}{2}(\delta_R + \delta_P)\) apparent diffusion coefficient. \(\hat{R}(x,q) \simeq \hat{G}(0,q)\frac{1}{1+K'}C_0\exp\left[-\frac{1}{2}(\delta_R + \delta_P)q^2 x\right]\) and \(\hat{P}(x,q) \simeq \hat{G}(0,q)\frac{K'}{1+K'}C_0\exp\left[-\frac{1}{2}(\delta_R + \delta_P)q^2 x\right]\) at zeroth order. In particular, it is impossible to access kinetics in the present approach when \(\delta_R = \delta_P\).

Both preceding situations can be used to derive the thermodynamic features of the reaction (2) by analyzing either the relative amplitudes of the biexponential decay (slow \(R \rightleftharpoons P\) exchange regime) or the average apparent diffusion coefficient (fast \(R \rightleftharpoons P\) exchange regime). Note that the former case is more informative than the latter one as it does not rely on any \(a\ priori\) knowledge of the \(R\) and \(P\) diffusion coefficients.[1] Nevertheless, none of these two simplifying situations is appropriate to reach the kinetic information contained in the \(\kappa_1 + \kappa_2\) term.\(^6\)

To progress in the identification of appropriate experimental conditions to extract the kinetic information from observing diffusion, one may notice from Eqs.(15) that, in the slow exchange regime, both eigenvalues, \(\lambda_+\) and \(\lambda_-\), do not contain the \(\kappa_1 + \kappa_2\) term.\(^6\)In the slow exchange regime, the amplitude ratio gives only access to the ratio \(\frac{\lambda_+}{\lambda_-}\), which depends on \(K'\).
at leading order ($\lambda_+ \approx -\delta_P q^2$ and $\lambda_- \approx -\delta_R q^2$). In contrast, $\lambda_-$ does contain the kinetic information at leading order in the fast exchange regime ($\lambda_+ \approx -\frac{1}{2}(\delta_P + \delta_R)q^2$ and $\lambda_- \approx -(\kappa_1 + \kappa_2)$). However, using Eqs. (21) and (23), we see that the associated amplitudes $R_-$ and $P_-$ scale as $\frac{[\delta_P - \delta_R]q^2}{\kappa_1 + \kappa_2}$, which is a very small term. In fact, the preceding asymptotic regimes suggest the $(\kappa_1 + \kappa_2) \approx (\delta_R - \delta_P)q^2$ regime to be the more appropriate to evidence kinetics by observing the spatial evolution of the diffusion profiles.\footnote{It is worth noting here that changing the applied voltage driving solutes motion is useful to access a suitable and large enough collection of spatial frequencies.} Indeed, both eigenvalues, $\lambda_+$ and $\lambda_-\text{,}$ then equally depend on chemistry and diffusion. Moreover, the corresponding amplitudes, $R_+$ and $R_-$ on one hand and $P_+$ and $P_-$ on the other hand, exhibit balanced values because $\lambda_+$ and $\lambda_-$ are at the closest in this regime (see Eqs. (20–23)).

Under the above defined conditions, one also has to take into account the concentration terms present in Eqs. (20–23). In particular, one should chose an appropriate concentration in $\mathcal{R}$ to fix $K'$ as close as possible to one. In fact, the $K' = 1$ value is optimal to simultaneously get similar amplitudes associated to the $\lambda_+$ and $\lambda_-$ eigenvalues, and thus to be able to extract the dynamic parameters sought for.\footnote{These conclusions are not markedly modified in the general case where Eqs. (16–19) rule the amplitudes of the exponential decays.}
References