

SUPPLEMENTARY INFORMATION

Two-peak approximation in kinetic capillary electrophoresis

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1. Derivation of ordinary differential equations for mass transfer in zones A* and C*

Mass transfer of A* and C* is described by the following equations:

$$\begin{aligned}(\partial_t + v_A \partial_x)A^* &= -k_+ A^* B + k_- C^* \\(\partial_t + v_C \partial_x)C^* &= k_+ A^* B - k_- C^*\end{aligned}\tag{S1}$$

where ∂_x and ∂_t are partial derivations by the spatial coordinate x and time t , respectively; A^* and C^* are linear concentrations of A* and C*, respectively (i.e. amounts of A* and C* per unit length of the reactor); $B = \text{const}$ is a volume concentration of B; and v_A and v_C are the velocities of A* and C*; k_+ and k_- are the rate constants of the forward and reversed processes in the following equilibrium:



Let us consider propagation patterns in a case of slow ($t_{\text{eq}} \gg t_{\text{sep}}$) or moderate-rate ($t_{\text{eq}} \sim t_{\text{sep}}$) equilibration. In this case, the two peaks, A* and C*, can be identified at the flanks of the label distribution (or the distribution of mixture of A* and C*). They move with velocities v_A and v_C , respectively (**Fig. S1**). As a result, the following transformations hold in zone A*:

$$\begin{aligned}\frac{d}{dt} \int_{x_a^-}^{x_a^+} A^* dx &= \int_{x_a^-}^{x_a^+} \frac{\partial A^*}{\partial t} dx + A^*(x_a^+) \frac{dx_a^+}{dt} - A^*(x_a^-) \frac{dx_a^-}{dt} = \\&= - \int_{x_a^-}^{x_a^+} \left(v_A \frac{\partial A^*}{\partial x} + k_+ B A^* - k_- C^* \right) dx + v_A \left[A^*(x_a^+) - A^*(x_a^-) \right] = \\&= -k_+ B \int_{x_a^-}^{x_a^+} A^* dx + k_- \int_{x_a^-}^{x_a^+} C^* dx\end{aligned}\tag{S3}$$

and

$$\begin{aligned} \frac{d}{dt} \int_{x_a^-}^{x_a^+} C^* dx &= \int_{x_a^-}^{x_a^+} \frac{\partial C^*}{\partial t} dx + C^*(x_a^+) \frac{dx_a^+}{dt} - C^*(x_a^-) \frac{dx_a^-}{dt} = \\ &= - \int_{x_a^-}^{x_a^+} \left(v_c \frac{\partial C^*}{\partial x} - k_+ B A^* + k_- C^* \right) dx + v_A [C^*(x_a^+) - C^*(x_a^-)] = \\ &= k_+ B \int_{x_a^-}^{x_a^+} A^* dx - k_- \int_{x_a^-}^{x_a^+} C^* dx - (v_c - v_A) C^*(x_a^+) \end{aligned} \quad (\text{S4})$$

Here, $x_a^-(t)$ and $x_a^+(t)$ are coordinates of the boundaries of zone A* (**Fig. S1**). To derive transformations (S3) and (S4), we used equations (S1) and the four following relations:

$$\int_{x_a^-}^{x_a^+} \frac{\partial A^*}{\partial x} dx = A^*(x_a^+) - A^*(x_a^-), \quad \int_{x_a^-}^{x_a^+} \frac{\partial C^*}{\partial x} dx = C^*(x_a^+) - C^*(x_a^-), \quad \frac{dx_a^\pm}{dt} = v_A, \quad C^*(x_a^-) = 0 \quad (\text{S5})$$

The last equation in (S5) follows from the fact that the concentration C^* vanishes at the left boundary of zone A*.

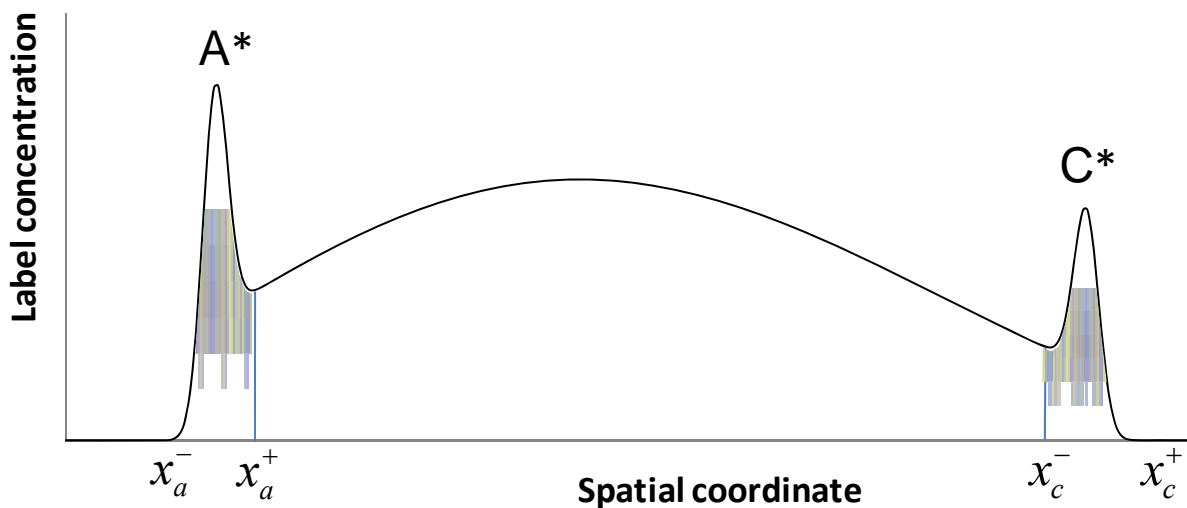


Figure S1. Spatial propagation pattern of the mixture of A* and C*. Their sum concentration coincides with that of the label. Areas corresponding to zones A* and C* are shaded. At $v_c > v_A$, zone C* is located to the right of zone A* (both zones move from left to right). Coordinates of the boundaries of zone A* are denoted by x_a^- and x_a^+ , whereas coordinates of the boundaries of zone C* are denoted by x_c^- and x_c^+ . At $k_- > Bk_+$, peak C* is lower than peak A*.

Transformations similar to (S3) and (S4) hold in zone C*:

$$\begin{aligned} \frac{d}{dt} \int_{x_c^-}^{x_c^+} A^* dx &= \int_{x_c^-}^{x_c^+} \frac{\partial A^*}{\partial t} dx + A^*(x_c^+) \frac{dx_c^+}{dt} - A^*(x_c^-) \frac{dx_c^-}{dt} = \\ & - \int_{x_c^-}^{x_c^+} \left(v_A \frac{\partial A^*}{\partial x} + k_+ B A^* - k_- C^* \right) dx + v_C \left[A^*(x_c^+) - A^*(x_c^-) \right] = \\ & - k_+ B \int_{x_c^-}^{x_c^+} A^* dx + k_- \int_{x_c^-}^{x_c^+} C^* dx - (v_C - v_A) A^*(x_c^-) \end{aligned} \quad (\text{S6})$$

and

$$\begin{aligned} \frac{d}{dt} \int_{x_c^-}^{x_c^+} C^* dx &= \int_{x_c^-}^{x_c^+} \frac{\partial C^*}{\partial t} dx + C^*(x_c^+) \frac{dx_c^+}{dt} - C^*(x_c^-) \frac{dx_c^-}{dt} = \\ & - \int_{x_c^-}^{x_c^+} \left(v_C \frac{\partial C^*}{\partial x} - k_+ B A^* + k_- C^* \right) dx + v_C \left[C^*(x_c^+) - C^*(x_c^-) \right] = \\ & k_+ B \int_{x_c^-}^{x_c^+} A^* dx - k_- \int_{x_c^-}^{x_c^+} C^* dx \end{aligned} \quad (\text{S7})$$

Here, $x_c^-(t)$ and $x_c^+(t)$ are coordinates of the left and right boundaries of zone C* (**Fig. S1**). To derive transformations (S4) and (S7), we again used equations (S1) and the following relations:

$$\int_{x_c^-}^{x_c^+} \frac{\partial A^*}{\partial x} dx = A^*(x_c^+) - A^*(x_c^-), \quad \int_{x_c^-}^{x_c^+} \frac{\partial C^*}{\partial x} dx = C^*(x_c^+) - C^*(x_c^-), \quad \frac{dx_c^\pm}{dt} = v_C, \quad A^*(x_c^+) = 0 \quad (\text{S8})$$

The last equation in (S8) follows from the fact that the concentration A^* approaches zero at the right boundary of zone C*.

Propagation patterns simulated with the exact solution of equations (S1) demonstrate the following behavior after introducing the initial plug containing compounds A^* and C^* . During time $\sim t_{\text{sep}}$, the peak corresponding to compound C^* moves out of zone A^* and the peak corresponding to compound A^* moves out of zone C^* . As a result, the distribution of C^* in zone A^* and the distribution of A^* in zone C^* become monotonic and can be approximated using power series:

$$C^*(x) = c_0 + c_1(x - x_a^-) + c_2(x - x_a^-)^2 + \dots \quad (x_a^- \leq x \leq x_a^+) \quad (\text{S9})$$

$$A^*(x) = a_0 + a_1(x - x_c^+) + a_2(x - x_c^+)^2 + \dots \quad (x_c^- \leq x \leq x_c^+) \quad (\text{S10})$$

Since $C^*(x)$ and $A^*(x)$ are smooth functions that vanish at x_a^- and x_c^+ , respectively, we have:

$$c_0 = 0, \quad c_1 = 0, \quad a_0 = 0, \quad a_1 = 0. \quad (\text{S11})$$

Therefore:

$$\int_{x_a^-}^{x_a^+} C^* dx = \frac{c_2}{3} (x_a^+ - x_a^-)^3 + \dots, \quad \int_{x_c^-}^{x_c^+} A^* dx = \frac{a_2}{3} (x_c^+ - x_c^-)^3 + \dots \quad (\text{S12})$$

By comparing expansions (S9) and (S10) to (S12) and taking into account only the leading terms in them, we obtain the following approximate relations:

$$C^*(x_a^+) \approx \frac{3}{x_a^+ - x_a^-} \int_{x_a^-}^{x_a^+} C^* dx, \quad A^*(x_c^-) \approx \frac{3}{x_c^+ - x_c^-} \int_{x_c^-}^{x_c^+} A^* dx \quad (\text{S13})$$

Given expressions (S13), relations (S3), (S4), (S6), and (S7) can be rewritten in the form:

$$\frac{dA_a^*}{dt} = -k_+ BA_a^* + k_- C_a^*, \quad \frac{dC_a^*}{dt} = k_+ BA_a^* - \left(k_- + \frac{3(v_C - v_A)}{W_a} \right) C_a^* \quad (\text{S14})$$

$$\frac{dA_c^*}{dt} = - \left(k_+ B + \frac{3(v_C - v_A)}{W_c} \right) A_c^* + k_- C_c^*, \quad \frac{dC_c^*}{dt} = k_+ BA_c^* - k_- C_c^* \quad (\text{S15})$$

Here, A_a^* and C_a^* are the total amounts of A* and C* in the zone of A*:

$$A_a^* = \int_{x_a^-}^{x_a^+} A^* dx, \quad C_a^* = \int_{x_a^-}^{x_a^+} C^* dx; \quad (\text{S16})$$

A_c^* and C_c^* are the total amounts of A* and C* in the zone of C*:

$$A_c^* = \int_{x_c^-}^{x_c^+} A^* dx, \quad C_c^* = \int_{x_c^-}^{x_c^+} C^* dx; \quad (\text{S17})$$

W_a and W_c are the widths of zones A* and C* that are defined by expressions:

$$W_a = x_a^+ - x_a^-, \quad W_c = x_c^+ - x_c^-. \quad (\text{S18})$$

By taking into account the following definitions for t_{sep} and t_{eq} :

$$t_{\text{sep}} = \frac{W}{|v_C - v_A|}, \quad t_{\text{eq}} = \frac{1}{Bk_+ + k_-}, \quad (\text{S19})$$

and by defining x_a^+ and x_c^- so that the widths of zones A* and C*, W_a and W_c , respectively, would coincide with the initial plug width W :

$$W_a = W, \quad W_c = W, \quad (\text{S20})$$

we finally obtain ordinary differential equations for mass transfer:

$$\frac{dA_a^*}{dt} = -k_+BA_a^* + k_-C_a^*, \quad \frac{dC_a^*}{dt} = k_+BA_a^* - \left(k_- + \frac{3}{t_{\text{sep}}}\right)C_a^* \quad (\text{S21})$$

$$\frac{dA_c^*}{dt} = -\left(k_+B + \frac{3}{t_{\text{sep}}}\right)A_c^* + k_-C_c^*, \quad \frac{dC_c^*}{dt} = k_+BA_c^* - k_-C_c^* \quad (\text{S22})$$

Equations (S21) and (S22) are identical to equations (6) and (7) in the main text. They allow a significant simplification of the mathematical part of the developed parameter-based method for finding k_+ and k_- . At the same time, this simplification results in only 10% relative errors in the rate constants as was shown by our detailed study (the results are shown in **Fig. 5** of the main text). This accuracy is acceptable for most kinetic studies of biomolecular interactions.

2. Solutions to ordinary differential equations for mass transfer in zones A* and C*

System of linear differential equations (S21) has a critical point located at the origin in the phase plane (A_a^* , C_a^*) (**Fig. S2**). Similarly, system of equations (S22) has a critical point located at the origin in the plane (A_c^* , C_c^*).

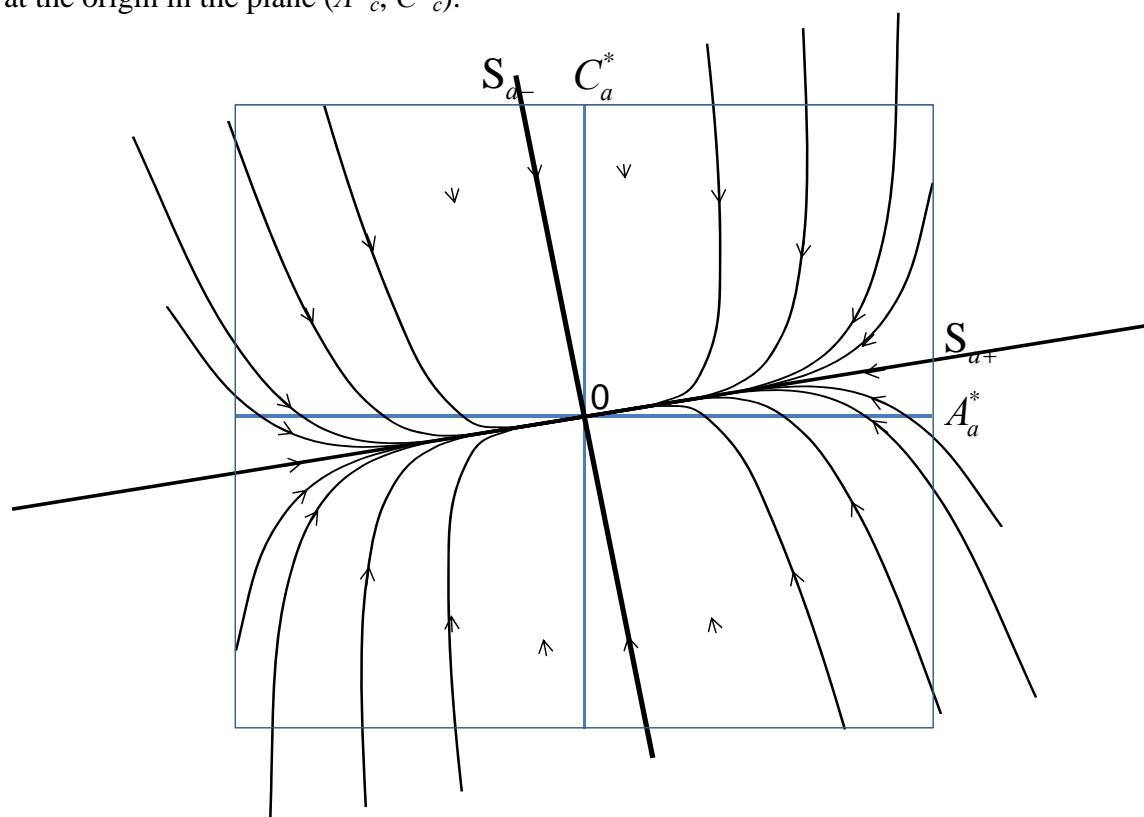


Figure S2. Qualitative phase portrait of system (S21). Its solutions are depicted by black lines in the plane (A_a^* , C_a^*). Arrows show directions in which time increases. The thick straight lines represent solutions that satisfy the first condition from (S27). Rays OS_{a+} and OS_{a-} intersect at a right angle if $K_d = B$. The ray OS_{a+} corresponds to the special solution (S34) and, therefore, $\tan \angle A_a^*OS_{a+} = \eta_a$. The phase portrait of system (S22) looks similar.

Let us denote eigenvalues of (S21) as $-\lambda_{a\pm}$ and eigenvalues of (S22) as $-\lambda_{c\pm}$. These eigenvalues satisfy quadratic equations that follow from (S21) and (S22):

$$\lambda_{a\pm}^2 - \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right) \lambda_{a\pm} + \frac{3Bk_+}{t_{\text{sep}}} = 0 \quad (\text{S23})$$

$$\lambda_{c\pm}^2 - \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right) \lambda_{c\pm} + \frac{3k_-}{t_{\text{sep}}} = 0 \quad (\text{S24})$$

Roots of (S23) and (S24) are determined by expressions:

$$\lambda_{a\pm} = \frac{1}{2} \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right) \pm \sqrt{\frac{1}{4} \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right)^2 - \frac{3Bk_+}{t_{\text{sep}}}} \quad (\text{S25})$$

$$\lambda_{c\pm} = \frac{1}{2} \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right) \pm \sqrt{\frac{1}{4} \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right)^2 - \frac{3k_-}{t_{\text{sep}}}} \quad (\text{S26})$$

Here, plus and minus signs in subscripts (at $\lambda_{a\pm}$ and $\lambda_{c\pm}$) correspond, respectively, to plus and minus signs in front of the radicals. Obviously, both eigenvalues $-\lambda_{a\pm}$ are real, distinct and negative. The same is true for eigenvalues $-\lambda_{c\pm}$. Therefore, both critical points are improper stable nodes.^{1,2} All solutions to system (S21) approach the point $A_a^* = 0$, $C_a^* = 0$ and all solutions to system (S22) approach the point $A_c^* = 0$, $C_c^* = 0$ (**Fig. S2**). In physical terms, such a behavior means that peaks A* and C* gradually decrease and eventually disappear with increasing time. As a result of this relaxation process, all compounds A* and C* will be distributed over the “bridge”. It is well known that solutions to a system of two ordinary differential equations can approach an improper stable node with distinct eigenvalues only in two directions.^{1,2} Therefore, we have at $t \rightarrow \infty$:

$$\frac{C_a^*(t)}{A_a^*(t)} = \eta_{a\pm} = \text{const}, \quad \frac{A_c^*(t)}{C_c^*(t)} = \eta_{c\pm} = \text{const}. \quad (\text{S27})$$

where the plus and minus signs in subscripts correspond to these different directions. Only non-negative values of $\eta_{a\pm}$ and $\eta_{c\pm}$ can describe physical processes since concentrations of A* and C* are non-negative quantities. This non-negativity condition for $\eta_{a\pm}$ and $\eta_{c\pm}$ fixes one possible direction in each node. Systems (S21) and (S22) have special solutions that satisfy (S27) at all $t \geq 0$ rather than at $t \rightarrow \infty$. They correspond to straight lines in the phase planes (A_a^*, C_a^*) and (A_c^*, C_c^*) (**Fig. S2**). To find such solutions, we have to substitute $C_a^*(t) = \eta_{a\pm} A_a^*(t)$ into system

(S21) and to substitute $A_c^*(t) = \eta_{c\pm} C_c^*(t)$ into system (S22). As a result, the following quadratic equations for $\eta_{a\pm}$ and $\eta_{c\pm}$ can be obtained:

$$k_- \eta_{a\pm}^2 - \left(Bk_+ - k_- - \frac{3}{t_{\text{sep}}} \right) \eta_{a\pm} - Bk_+ = 0 \quad (\text{S28})$$

and

$$Bk_+ \eta_{c\pm}^2 - \left(k_- - Bk_+ - \frac{3}{t_{\text{sep}}} \right) \eta_{c\pm} - k_- = 0 \quad (\text{S29})$$

Roots of these equations are determined by expressions

$$\eta_{a\pm} = \frac{1}{k_-} \left\{ \frac{1}{2} \left(Bk_+ - k_- - \frac{3}{t_{\text{sep}}} \right) \pm \sqrt{\frac{1}{4} \left(Bk_+ - k_- - \frac{3}{t_{\text{sep}}} \right)^2 + Bk_+ k_-} \right\} \quad (\text{S30})$$

and

$$\eta_{c\pm} = \frac{1}{Bk_+} \left\{ \frac{1}{2} \left(k_- - Bk_+ - \frac{3}{t_{\text{sep}}} \right) \pm \sqrt{\frac{1}{4} \left(k_- - Bk_+ - \frac{3}{t_{\text{sep}}} \right)^2 + Bk_+ k_-} \right\} \quad (\text{S31})$$

The plus signs in front of the radicals obviously correspond to positive roots $\eta_a \equiv \eta_{a+}$ and $\eta_c \equiv \eta_{c+}$. Expressions (S30) and (S31) for these roots can be rewritten as follows:

$$\eta_a = \frac{Bk_+ - \lambda_a}{k_-} \geq 0, \quad \eta_c = \frac{k_- - \lambda_c}{Bk_+} \geq 0, \quad (\text{S32})$$

where $\lambda_a \equiv \lambda_{a-}$ and $\lambda_c \equiv \lambda_{c-}$. Given expression (S25) and (S26) for λ_{a-} and λ_{c-} , we also have:

$$\lambda_a = \Omega - \sqrt{\Omega^2 - \frac{3Bk_+}{t_{\text{sep}}}}, \quad \lambda_c = \Omega - \sqrt{\Omega^2 - \frac{3k_-}{t_{\text{sep}}}}, \quad \Omega \equiv \frac{1}{2} \left(Bk_+ + k_- + \frac{3}{t_{\text{sep}}} \right). \quad (\text{S33})$$

Finally, direct substitutions of expressions:

$$A_a^* = N_a \exp(-\lambda_a t), \quad C_a^* = \eta_a N_a \exp(-\lambda_a t), \quad (\text{S34})$$

and

$$A_c^* = \eta_c N_c \exp(-\lambda_c t), \quad C_c^* = N_c \exp(-\lambda_c t) \quad (\text{S35})$$

into equations (S21) and (S22), respectively, show that (S34) and (S35) are solutions to these equations. Here, N_a and N_c are constant coefficients that relate to initial amounts A_0^* and C_0^* of compounds A* and C* in the plug:

$$N_a + \eta_c N_c = A_0^*, \quad \eta_a N_a + N_c = C_0^*. \quad (\text{S36})$$

Obviously, solutions (S34) and (S35) also satisfy conditions (S27) with positive values of constants. These solutions are represented by the ray OS_{a+} in **Fig. S2** and by a similar ray (that should be denoted as OS_{c+}) in the phase portrait of system (S22).

If $t_{sep} \ll t_{eq}$, we have from definitions (S33) for Ω and (S19) for t_{sep} and t_{eq} :

$$\frac{3Bk_+}{t_{sep}\Omega^2} = \frac{12Bk_+}{t_{sep}\left(Bk_+ + k_- + \frac{3}{t_{sep}}\right)^2} < \frac{12t_{sep}}{t_{eq}\left(\frac{t_{sep}}{t_{eq}} + 3\right)^2} < \frac{4t_{sep}}{3t_{eq}} \ll 1 \quad (S37)$$

$$\frac{3k_-}{t_{sep}\Omega^2} = \frac{12Bk_-}{t_{sep}\left(Bk_+ + k_- + \frac{3}{t_{sep}}\right)^2} < \frac{12t_{sep}}{t_{eq}\left(\frac{t_{sep}}{t_{eq}} + 3\right)^2} < \frac{4t_{sep}}{3t_{eq}} \ll 1 \quad (S38)$$

In this case, expressions (S33) can be expanded in small parameter t_{sep}/t_{eq} . As a result, we obtain the following asymptotic relations (to the first order in t_{sep}/t_{eq}):

$$\lambda_a = \Omega - \Omega \sqrt{1 - \frac{3Bk_+}{t_{sep}\Omega^2}} \approx \Omega - \Omega \left(1 - \frac{3Bk_+}{2t_{sep}\Omega^2}\right) = \frac{3Bk_+}{2t_{sep}\Omega} = Bk_+ \left(1 + \frac{t_{sep}}{3t_{eq}}\right)^{-1} \approx Bk_+ \left(1 - \frac{t_{sep}}{3t_{eq}}\right) \quad (S39)$$

$$\lambda_c = \Omega - \Omega \sqrt{1 - \frac{3k_-}{t_{sep}\Omega^2}} \approx \Omega - \Omega \left(1 - \frac{3k_-}{2t_{sep}\Omega^2}\right) = \frac{3k_-}{2t_{sep}\Omega} = k_- \left(1 + \frac{t_{sep}}{3t_{eq}}\right)^{-1} \approx k_- \left(1 - \frac{t_{sep}}{3t_{eq}}\right). \quad (S40)$$

Therefore, λ_a and λ_c approach Bk_+ and k_- , respectively, if the ratio t_{sep}/t_{eq} approaches zero. At small values of t_{sep}/t_{eq} we obtain:

$$\lambda_a \approx Bk_+, \quad \lambda_c \approx k_- \quad (t_{sep}/t_{eq} \ll 1), \quad (S41)$$

Relations (S32) – (S35) and (S41) are identical to relations (8) – (14) in the main text.

Given (S25) and (S26), the following inequalities are fulfilled for the absolute values of eigenvalues:

$$\frac{\lambda_{a+}}{\lambda_{a-}} > 1, \quad \frac{\lambda_{c+}}{\lambda_{c-}} > 1. \quad (S42)$$

Therefore, using a general theory of dynamic systems,^{1,2} we can conclude that all (but two) solutions to system (S21) approach the critical point of (S21) in the same direction as the special solution (S34) (**Fig. S2**). Similarly, all (but two) solutions to system (S22) approach the critical point of (S22) in the same direction as the special solution (S35). Thus, solutions (S34) and (S35) are stable. Actually, the ratios (S37) are significantly larger than unity (for example, they are more than 5.83 at $K_d = B$). Because of this fact, the convergence of other solutions to the special solutions (S34) and (S35) occurs relatively quickly (**Fig. S2**). As a result, special solutions can be considered good candidates to describe processes of relaxation of peaks A^* and C^* . One could expect that they describe the relaxation that occurs at the maximum speed and, therefore, takes place in reality.

3. Determination of the rate constants k_+ and k_- based on the total amounts of the mixture of A^* and C^* in zones A^* and C^*

Taking into account (S34) and (S35), we have:

$$L_a(t_{a1}) \equiv A_a^* + C_a^* = (1 + \eta_a) N_a \exp(-\lambda_a t_{a1}), \quad L_c(t_{c1}) \equiv A_c^* + C_c^* = (1 + \eta_c) N_c \exp(-\lambda_c t_{c1}), \quad (\text{S43})$$

$$L_a(t_{a2}) \equiv A_a^* + C_a^* = (1 + \eta_a) N_a \exp(-\lambda_a t_{a2}), \quad L_c(t_{c2}) \equiv A_c^* + C_c^* = (1 + \eta_c) N_c \exp(-\lambda_c t_{c2}). \quad (\text{S44})$$

Here L_a and L_c are the total amounts of the label (or the mixture of A* and C*) in zones A* and C*, respectively; t_{a1} and t_{a2} are two different times at which L_a is measured; similarly, t_{c1} and t_{c2} are two (other) different times at which L_c is measured. It is obvious from relations (S43) and (S44) that:

$$\frac{L_a(t_{a1})}{L_a(t_{a2})} = \exp[\lambda_a(t_{a2} - t_{a1})], \quad \frac{L_c(t_{c1})}{L_c(t_{c2})} = \exp[\lambda_c(t_{c2} - t_{c1})]. \quad (\text{S45})$$

By solving (S45) with respect to λ_a and λ_c , we obtain the following expressions for them in terms of the measurable quantities (L_a , L_c , t_{a1} , t_{a2} , t_{c1} , t_{c2}):

$$\lambda_a = \frac{1}{t_{a2} - t_{a1}} \ln \frac{L_a(t_{a1})}{L_a(t_{a2})}, \quad \lambda_c = \frac{1}{t_{c2} - t_{c1}} \ln \frac{L_c(t_{c1})}{L_c(t_{c2})}. \quad (\text{S46})$$

They coincide with relations (17) from the main text. On the other hand, λ_a and λ_c can be expressed in terms of rate constants, k_+ and k_- , using (S33). Remarkably, to determine k_+ and k_- , we actually do not need to solve algebraic equations (S33) containing radicals. The rate constants can be easily expressed in terms of λ_a and λ_c , using relations (S23) and (S24). The latter form a system of linear algebraic equations with respect to k_+ and k_- . By adding up (S23) and (S24), we obtain an equation that can be readily solved with respect to $Bk_+ + k_- \equiv 1/t_{\text{eq}}$. As a result,

$$\frac{1}{t_{\text{eq}}} \equiv Bk_+ + k_- = \frac{3(\lambda_a + \lambda_c) - t_{\text{sep}}(\lambda_a^2 + \lambda_c^2)}{3 - t_{\text{sep}}(\lambda_a + \lambda_c)}. \quad (\text{S47})$$

Then, by solving (S23) and (S24) with respect to Bk_+ and k_- , we finally obtain:

$$Bk_+ = \left(1 + \frac{t_{\text{sep}}}{3t_{\text{eq}}}\right) \lambda_a - t_{\text{sep}} \lambda_a^2, \quad k_- = \left(1 + \frac{t_{\text{sep}}}{3t_{\text{eq}}}\right) \lambda_c - t_{\text{sep}} \lambda_c^2. \quad (\text{S48})$$

Here, $1/t_{\text{eq}}$ is presumed to be expressed in terms of λ_a and λ_c , using (S47). Solutions (S47) and (S48) coincide with relations (18) – (20) in the main text. It is obvious from (S46) – (S48) that the rate constants are completely determined by $L_a(t_{a1})/L_a(t_{a2})$ and $L_c(t_{c1})/L_c(t_{c2})$, i.e. by the ratios of the amounts of the label (or the mixture of A* and C*) measured at two different times in zones A* and C*. Total amounts L_a and L_c in (S46) can be replaced by total signals L_{fa} and

L_{fc} from zones A* and C*, respectively, as it is explained in the main text. Then ratios $L_{fa}(t_{a1})/L_{fa}(t_{a2})$ and $L_{fc}(t_{c1})/L_{fc}(t_{c2})$ can be calculated using the areas of corresponding zones in spatial or temporal propagation patterns of the label (or the mixture of A* and C*) (**Fig. S1**), given the following relations between these areas:

$$\int_{x_a^-}^{x_a^+} L_{fa}(x, t_{a1}) dx = v_A \int_{t_a^+}^{t_a^-} L_{fa}(x_1, t) dt, \quad \int_{x_c^-}^{x_c^+} L_{fc}(x, t_{c1}) dx = v_C \int_{t_c^+}^{t_c^-} L_{fc}(x_1, t) dt, \quad (\text{S49})$$

$$\int_{x_a^-}^{x_a^+} L_{fa}(x, t_{a2}) dx = v_A \int_{t_a^+}^{t_a^-} L_{fa}(x_2, t) dt, \quad \int_{x_c^-}^{x_c^+} L_{fc}(x, t_{c2}) dx = v_C \int_{t_c^+}^{t_c^-} L_{fc}(x_2, t) dt \quad (\text{S50})$$

Here, t_a^+ and t_a^- are moments of time in the temporal propagation pattern of zone A* corresponding to the boundaries x_a^+ and x_a^- in its spatial propagation pattern. Similarly, t_c^+ and t_c^- are moments of time in the temporal propagation pattern of zone C* corresponding to the boundaries x_c^+ and x_c^- in its spatial propagation pattern. Obviously, $t_a^+ < t_a^-$ and $t_c^+ < t_c^-$ since right boundaries x_a^+ and x_c^+ reach the detector earlier than corresponding left boundaries x_a^- and x_c^- . The above considerations are based on definitions of the right and left boundaries (x_a^+ and x_c^-) for zones A* and C*, respectively, that lead to relations (S20). These “inner” boundaries of zones A* and C* could be defined in some other way, for example, as coordinates of the local minimums in the concentration of the label (or the mixture of A* and C*) (**Fig. S1**). These minimums can be found at the flanks of the “bridge” connecting zones A* and C*. Let us assume that widths W_a and W_c of such redefined zones A* and C* remain approximately constant with time but differ significantly from W . Then, the above results will remain valid after the following modification. If $W_a \approx W_c$ one should just use W_a or W_c (instead of W) in definition (S19) for t_{sep} . In more general case, when W_a and W_c vary considerably, different separation times:

$$t_a = \frac{W_a}{|v_C - v_A|}, \quad t_c = \frac{W_c}{|v_C - v_A|} \quad (\text{S51})$$

should be used in zones A* and C*, respectively. Thus, all above results related to zone A* will hold true if t_{sep} is replaced with t_a in the corresponding equations. Similarly, results related to zone C* will hold true if t_{sep} is replaced with t_c . However, expressions (S47) and (S48) for the rate constants cannot be modified in such a way. Indeed, to derive (S47) and (S48), we used the fact that both (S23) and (S24) contain the same parameter t_{sep} . It should be replaced with t_a (in equation (S23) or with t_c (in equation (S24)), if the widths W_a and W_c of zones A* and C* are different. After such a modification of equations (S23) and (S24), their solutions cannot be found by the simple way described. Instead, one should apply Cramer’s rule.³ As a result, expressions for Bk_+ and k_- become more cumbersome. Moreover, this procedure requires complex processing of experimental data to identify boundaries of zones A* and C*.

References

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- 3 Leon, S. J. *Linear Algebra with Applications* (7th ed.), Pearson Prentice Hall, 2006 (544 pages).