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Micromixing

Quantitative Characterization of Micromixing Based on Uniformity and Overlap**

*Victor Okhonin, Alexander P. Petrov, Svetlana M. Krylova, and Sergey N. Krylov**

Micromixing is "interpenetration" of solutions by molecular diffusion and field-induced differential mobility of molecules;[1,2] it is neceessary for any homogeneous reaction to occur. In macroreactors, micromixing occurs after macromixing (breaking macrovolumes of solutions into microvolumes by mechanical agitation).[3–6] In microreactors, where mechanical agitation is technically difficult,[7] micromixing can be a sole means of mixing.[8,9] Better mixing is associated with better uniformity of reactant distribution through the reactor and greater spatial overlap of the reactants. Therefore, the characterization of micromixing requires a quantitative parameter that takes into account both the uniformity and the overlap. Multiple quantitative measures for macromixing have been developed and comprehensively reviewed over decades.[3–6] They all are stochastic functions that are not applicable to micromixing, which is driven solely by the deterministic processes of diffusion and/or differential mobility. Measures of micromixing, such as coefficient of variation, quantitative overlap, and some empirical functions, do not take into consideration both the uniformity and the overlap.[10–12] Herein, we introduce nine axioms of micromixing that must be satisfied by any qualitative attribute of the quality of micromixing and design a satisfying quantitative attribute, which we term "micromixing extent" (ME). ME takes into account both the uniformity of reactant distributions through the reactor and the spatial overlap of the reactants; it changes from zero (for no mixing) to one (for complete mixing). Importantly, ME is a general quantitative measure that can be applied to all types of mixtures that are created solely by diffusion and/or differential mobility for which distributions of the solutes through the reservoir can be calculated. For example, we use ME to characterize the quality of micromixing in a capillary microreactor, a type of reactor in which good-quality mixing is difficult to achieve.[13] Our approach for characterization of the quality of micromixing can be used for the optimization of mixing in microreactors by simply maximizing the value of ME.

Considering the generality of our approach and its applicability to reacting and non-reacting molecules, we use terms "solute" and "reservoir" instead of "reactant" and "reactor", respectively. We also use the words mixing and micromixing interchangeably.

In micromixing, the exact solute distributions through the mixture can be easily calculated using deterministic equations.[14] The present work was inspired by the insight that the knowledge of solute distributions creates the foundation for a quantitative description of the quality of micromixing based on the uniformity and the overlap. We first discuss the uniformity and the overlap using common sense. In general, the goal of micromixing is to achieve as homogeneous a molecular "amalgamation" of mixed solutes as possible. In an ideal mixture, every solute uniformly fills the entire volume of the reservoir, and the uniformity is a sufficient property to describe the quality of mixing. This is not the case if mixing is non-ideal, as illustrated in Figure 1a. In Figure 1a1 and Figure 1a2, two solutes are mixed in ways that keep their uniformity of distributions identical. However, it is clear that the mixing is better in Figure 1a2 owing to a better overlap of the two solutes. On the other hand, the overlap on its own is inadequate to describe mixing; two solutes may be completely overlapping but have poor mixing if the solutes have non-uniform concentration distributions. Figure 1b illustrates situations in which the solutes overlap throughout the reservoir, but the mixing quality is different as a result of variations in uniformity. The uniformity is greater for the blue solute in Figure 1b2 than in Figure 1b1, resulting in superior mixing in Figure 1b2.

Given that the quality of micromixing depends on both the uniformity of solute distributions and the spatial overlap of the solutes, our goal was to design a quantitative attribute that ranges from zero to one and describes the quality of micromixing using both criteria. This attribute should be defined by the distributions of solutes throughout the reservoir or, in other words, by the dependence of solute concentrations on their spatial positions in the reservoir at any given time. We coin the term "micromixing extent" (ME) to name this attribute.

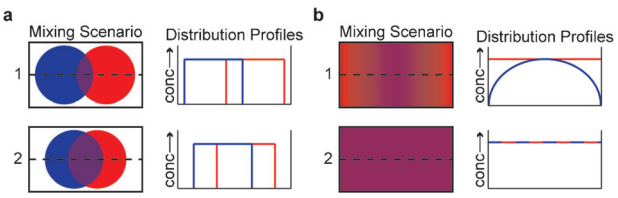

Figure 1. Graphical illustration of mixing red and blue solutes in a two-dimensional reservoir. a) Scenarios 1 and 2 have equal uniformities but different overlaps. b) Scenarios 1 and 2 have equal overlap but different uniformities. The corresponding concentration distribution profiles are shown next to each mixing scenario.

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To deduce a suitable mathematical definition of ME, we first developed a set of axioms that the definition must satisfy in order to comply with the common-sense understanding of molecular mixing. We define nine such common-sense axioms, which are summarized and graphically illustrated in Table 1. Axiom 1 demands that a maximum value of ME = 1 be only achieved when all solutes are distributed uniformly through the entire volume of the reservoir; this is the case in an ideal mixture. Axiom 2 demands that the minimum value of ME = 0 be achieved when the volume in which all solutes are present at the same time is equal to zero. Intuitively, there is an infinite number of ways in which solutes can be mixed to lead to the minimum ME = 0. Axiom 3 demands that ME does not depend on the multiplication of any concentration distribution by any non-zero constant. This axiom makes ME independent of the absolute values of solute concentrations but dependent on their concentration profiles, which makes ME a function of the mixing procedure only. Clearly, this requirement is essential for axiom 1 to be satisfied; otherwise solutes that are uniformly distributed throughout the reservoir but with different concentrations could not have ME = 1. Axiom 4 demands that ME of any set of solutes be less than or equal to that of any subset of the same solutes. This axiom originates from the understanding that increasing the number of solutes can only complicate their mixing. Axiom 5 is related to axiom 4; it demands that adding a new solute with a concentration distribution identical to that of a solute that is already present does not change ME. The need for such an axiom is obvious if we consider that the added solute could be identical to one which is present with the same distribution. In such a case, the addition of a solute would be identical to multiplying the concentration profile of this solute by a constant greater than 1 (see axiom 3). Axiom 6 demands that the diffusion of solutes with identical diffusion coefficients in a closed reservoir leads to improved ME. This axiom follows from our fundamental understanding that diffusion is a natural mechanism of mixing. If the diffusion coefficients are equal, the uniformity increases as the reagents diffuse throughout the reservoir, and the spatial overlap also increases as the solutes diffuse towards each other. The combined effect of improved uniformity and overlap leads to an increase in ME. If the diffusion coefficients are greatly different, a solute with a large diffusion coefficient can temporarily diffuse away from the location of solutes with low diffusion coefficients, thus potentially leading to a temporarily decreased overlap and ME. This phenomenon is illustrated in Figure S1 in the Supporting Information. Axiom 7 demands that ME does not change during hydrodynamic transfer of solutes if there is no diffusion, differential mobility, or flow through the solute boundaries. In the corresponding illustration in Table 1, a highly ordered structure is shown on the left; on the right, this structure is destroyed by hydrodynamic mass transfer, producing a complex pattern. This axiom again follows from the definition of micromixing that it is driven by the nonstochastic processes of diffusion and/or differential mobility. In mathematical terms, axiom 7 requires that ME be an integral of motion. Axiom 8 states that when the concentration profiles of all solutes are composed of rectangular shapes, with the concentration being either zero or a nonzero constant (a single constant for a single solute), ME can be determined from the quotient of the volumes occupied by all the solutes and the total volume of the reservoir. Axiom 9 demands that ME vary with the void volume of the reservoir. It should be noted that, in general, the boundaries of the reservoir are not physical but virtual (see examples below). In qualitative terms, by extending the volume of the reservoir without changing the solute distributions, the solutes occupy a smaller part of the total volume, resulting in a decrease in uniformity and ME. The nine deduced axioms were used for finding a suitable mathematical definition of ME.

After designing and analyzing a number of potential mathematical constructs to define ME, we found one that incorporates both the uniformity and overlap and satisfies the nine axioms (as shown below and proven in the Supporting

Table 1: Axioms for ME and their graphic illustrations

Illustration[a]	Axiom
$ME_1 < 1$ $ME_2 = 1$	1. Maximum ME = 1 can be achieved in a single way; all solutes are distributed uniformly throughout the entire reservoir.
$ME_1 = 0$ $ME_2 = 0$	2. Minimum ME = 0 can be achieved in a variety of ways; when the solutes do not spatially overlap.
$ME_1 = ME_2$	3. ME should not depend on the multiplication of a concentration distribution by a non-zero constant.
$ME_1 > ME_2$	4. ME for all solutes should be less than or equal to ME of any of their subsets.
$ME_1 = ME_2$	5. Adding a new solute with concentration distribution identical to that of an already present solute should not change ME.
$ME_1 < ME_2$	6. Diffusion of solutes with identical diffusion coefficients in a closed reservoir leads to an increase in ME.
$ME_1 = ME_2$	7. ME should not change upon the hydrodynamic transfer of solutes without diffusion, differential mobility, or flow through solute boundaries.
$ME = (v_1+v_2)/V$, V, v_1, v_2	8. If all concentration distributions are rectangular and take a single value for a single solute, ME is the quotient of the sum of mixture volumes where all solutes are present and the total reservoir's volume.
$ME_1 = 2ME_2$	9. If empty volume is added to the reservoir without changing distributions of solutes, mixing efficiency will change in inverse proportion to the total volume of the reservoir.

[a] Illustrations 7 and 9 depict closed two-dimensional reservoirs; the two axes correspond to two spatial coordinates. All other illustrations are shown for one-dimensional mixtures; the horizontal axes correspond to a spatial coordinate and the vertical axes correspond to the solute concentration. The arrowed lines in illustration 8 indicate the respective one-dimensional volumes.

Information). Equation (1) defines ME using this mathematical construct:

$$\mathrm{ME} = \frac{1}{V}\int_V \min\left(C_1(\vec{r},t)\frac{\frac{1}{V}\int_V C_1(\vec{r},t)d\vec{r}}{\frac{1}{V}\int_V C_1^2(\vec{r},t)d\vec{r}}, \ldots C_n(\vec{r},t)\frac{\frac{1}{V}\int_V C_n(\vec{r},t)d\vec{r}}{\frac{1}{V}\int_V C_n^2(\vec{r},t)d\vec{r}} \right) d\vec{r} \quad (1)$$

where V is the volume of an m-dimensional reservoir ($m = 1,2,3$), $\vec{r}$ is a vector of the spatial coordinate, t is the time, $C_i(\vec{r},t)$ is the concentration of the ith solute as a function of $\vec{r}$ and t, and n is the total number of solutes. To satisfy axiom 4, "min" is defined as a function of $\vec{r}$ and t, and its value is equal to the smallest of the n possible values of $C_i(\vec{r},t)\int C_i(\vec{r},t)d\vec{r}/C_i^2(\vec{r},t)d\vec{r}$. Note that the integral of a function over the reservoir volume divided by the total volume of integration in Equation (1) is equivalent to the spatial average of this function. We use $\bar{C}_i$ to designate the spatial average of the concentration of the ith component. Equation (1) can thus be written in a simplified form [Eq. (2)]:

$$\mathrm{ME} = \overline{\min\left(C_1\bar{C}_1/\overline{C_1^2}, \ldots C_n\bar{C}_n/\overline{C_n^2}\right)} \quad (2)$$

Equations (1) and (2) define ME as a spatial average, thus making it a function of time only. It should be noted that the effect of $\overline{C_i^2}$ in the denominator is to normalize the effect of solutes with varying concentrations (as required by axiom 3). The mathematical proof that our definition of ME satisfies the nine axioms and the consideration of alternative definitions are presented in the Supporting Information.

Herein we show that our definition of ME incorporates both the uniformity of solute distributions through the reservoir and the spatial overlap of solutes. In general, the uniformity and the overlap depend on each other and cannot be presented as two separate terms in Equation (1). However, for the two extreme cases considered below, they can be separated into two terms. In the first case, the overlap is completely excluded from consideration by having a single solute, with concentration distribution C, in the reservoir. The ME of a single solute is dependent on its distribution uniformity; accordingly, using Equation (2) for a single solute, we can define the quantitative meaning of uniformity, called quantitative uniformity [QU, Eq. (3)]:

$$\mathrm{QU} = \mathrm{ME}_{\text{single solute}} = \overline{\min\left(C\bar{C}/\overline{C^2}\right)} = \overline{C\bar{C}/\overline{C^2}} = \bar{C}^2/\overline{C^2} \quad (3)$$

The two consequent simplifications in Equation (3) are based, respectively, on two mathematical rules: the minimum of a single value is the value itself, and the average of a single value is the value itself. QU, as defined by Equation (3), ranges from 0 to 1, which is a known property of this function, widely used in mathematical statistics. QU is equal to 0 in the case of "singularity", that is, when all solute molecules are present at a single spatial point, which is experimentally unattainable. QU is equal to 1 when the concentration of the solute in every point of the reservoir is equal to the average concentration; this situation corresponds to a uniformly distributed solute. Figure S2 in the Supporting Information illustrates QU for a number of different concentration profiles.

Since the term QU is now quantitatively defined, we can consider the second extreme case, in which multiple solutes are distributed through the reservoir with the identical uniformity of $\mathrm{QU} = \bar{C}^2/\overline{C^2}$ allowing Equation (2) to be rearranged, leading to Equation (4):

$$\mathrm{ME}_{\text{identical QU}} = \mathrm{QU}\overline{\min\left(C_1/\bar{C}_1, \ldots C_n/\bar{C}_n\right)} \quad (4)$$

In deriving Equation (4), we use the mathematical rule that if each term of a series is multiplied by the same positive coefficient, the minimum of the new series is equal to this coefficient multiplied by the minimum of the original series. The second term at the right-hand side of Equation (4) defines the quantitative meaning of the overlap or simply quantitative overlap (QO), which does not include the QU term [Eq. (5)]:

$$\mathrm{QO}_{\text{identicalgtQU}_i\text{gtforgti=1,...n}} = \overline{\min\left(C_1/\bar{C}_1, \ldots C_n/\bar{C}_n\right)} \quad (5)$$

Note that this QO obtained as a degenerate case of ME is equivalent to QO recently introduced by using a different approach.[11]

Figure 2 illustrates examples of micromixing of three solutes that are characterized by identical values of $\mathrm{QU} = 0.25$ but different QO and, accordingly, different ME. Figure 2a,b demonstrates $\mathrm{QO} = 0$, as there is no volume occupied by all three solutes. The values of QO and ME become nonzero when the volume in which all three solutes are present becomes nonzero. This situation is illustrated in Figure 2c where $\mathrm{QO} = 1$, as the three solutes occupy the same volume, but $\mathrm{ME} < 1$ as the solutes do not uniformly occupy the entire volume of the reservoir. More details on QU and QO can be found in the Supporting Information.

We have shown above that, in addition to satisfying the nine axioms, our definition of ME [Eqs. (1) and (2)] accounts for both the uniformity and the overlap. Practically, to calculate ME the concentrations of all solutes must first be determined as a function of spatial coordinate for any given time, $C_i(\vec{r},t)$, which can be done by analytically solving equations of mass transfer by diffusion field-induced differential mobility of the solutes. We have found analytical solutions for a relatively complex case of a capillary micro-

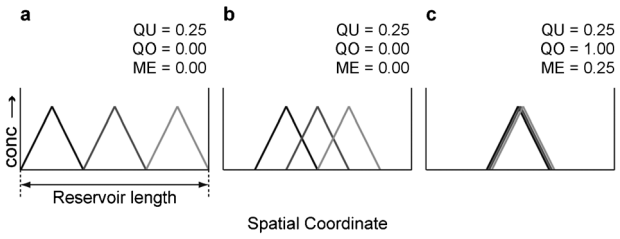

Figure 2. Graphical illustration of mixing three solutes in a one-dimensional reservoir. Quantitative uniformities are equal in every panel and between the panels. The quantitative overlap and, accordingly, micromixing extent, are zero in (a) and (b) and greater than zero in (c).

reactor with sequential injections of the solutes.[15] Alternatively, the concentrations of all solutes as a function of spatial coordinates for any given time can be found numerically. Custom-made software and a numerical approach described in sections 4.2.4–4.2.6 of reference [14] were used in an example below. COMSOL, a user-friendly physics software, makes it possible to find the concentrations of solutes micromixed by diffusion even without writing differential equations.[16]

Then, the boundaries of the reservoir should be defined to allow the integration over the reservoir volume. Finally, Equation (1) is used to calculate ME. To illustrate the use of ME, we simulated two examples of the micromixing of two solutes in an open-capillary microreactor by 1) a field-induced differential mobility along the reactor axis and 2) diffusion (see the Supporting Information).

Finally, we demonstrated that ME is not only a theoretically sound parameter, but it also has a practically useful predictive property. The major reason for mixing molecules in chemistry is to facilitate efficient reaction to, for example, achieve high product yield. We thus tested if ME could be used to optimize micromixing with the goal of increasing product yield. ME was calculated for recently described experiments.[11] The experiments included mixing and hybridizing two strands of complimentary DNA inside a capillary. Briefly, the two DNA solutions were sequentially injected into a capillary by pressure pulses to form plugs with interpenetrating parabolic profiles. After injection, DNA strands mixed by transverse diffusion, and the double-stranded DNA (dsDNA) hybrid was formed. The amount of dsDNA formed was then determined, and the relative product yield was calculated.

To determine ME for these experiments, we first calculated the concentrations of the two DNA strands after mixing as functions of the position in a capillary. The calculations were performed by numerically solving the equations of mass transfer as described in sections 4.2.4–4.2.6 of reference [14]. Custom-made software, Excel with a DLL library written in Object Pascal,[17] was used for calculations (available for download from the "KCE tools" table in the Research section of www.chem.yorku.ca/profs/krylov). The output of calculations was the concentrations of the two DNA strands after mixing as functions of their position in the capillary. These concentrations were then used to calculate ME with Equation (1). For convenience, the calculation of ME was performed in the same Excel spreadsheet. The relative amount of the product was found to correlate well with ME (Figure 3), thus suggesting that ME can be used to maximize the product yield of bimolecular reactions in microreactors by maximizing ME.

To conclude, we introduced a quantitative attribute of micromixing, termed ME, that takes into account two mixing criteria: the uniformity of solute distributions through the reservoir's volume and the spatial overlap of the solutes. According to our definition, ME is a mathematical function that depends only on concentration profiles of solutes in the reservoir; therefore, this parameter is applicable to all kinds of mixtures, including liquid mixtures and solid mixtures, which are created by diffusion of field-induced differential mobility, and for which concentration profiles of solutes can be calculated. Our newly introduced approach for quantitative characterization of the quality of micromixing will allow for the optimization of micromixing by simply maximizing ME.

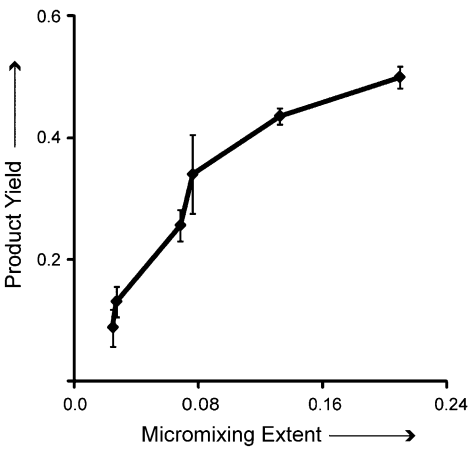

Figure 3. Dependence of product yield on ME. See text for details.

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Keywords: differential mobility · diffusion · micromixing extent · microreactors · product yield

[1] S. M. Krylova, V. Okhonin, C. J. Evenhuis, S. N. Krylov, *TrAC Trends Anal. Chem.* **2009**, *28*, 987 – 1010.
[2] J. Zhang, J. Hoogmartens, A. Van Schepdael, *Electrophoresis* **2010**, *31*, 65 – 73.
[3] P. V. Danckwerts, *Appl. Sci. Res.* **1952**, *3*, 279 – 296.
[4] J. M. Ottino, *The Kinematics of Mixing: Stretching, Chaos, and Transport (Cambridge Texts in Applied Mathematics)*, Cambridge University Press, Cambridge, **1989**.
[5] K. Ogawa, *Chemical Engineering: A New Perspective*, Elsevier, Amsterdam, **2007**, pp. 21 – 80.
[6] *Micro and Macro Mixing* (Eds.: H. Bockhorn, D. Mewes, W. Peukert, H.-J. Warnecke), Springer, Berlin, **2009**.
[7] *Microfluidic Technologies for Miniaturized Analysis Systems* (Eds.: S. Hardt, F. Schönfeld), Springer Science + Business Media, New York, **2007**.
[8] H. Okamoto, T. Ushijima, O. Kitoh, *Chem. Eng. J.* **2004**, *101*, 57.
[9] T. Kirner, J. Albert, M. Günther, G. Mayer, K. Reinhäckel, J. M. Köhler, *Chem. Eng. J.* **2004**, *101*, 65 – 74.
[10] J. Aubin, D. F. Fletcher, J. Bertrand, C. Xuereb, *Chem. Eng. Technol.* **2003**, *26*, 1262 – 1270.
[11] A. P. Petrov, B. J. Dodgson, L. T. Cherney, S. N. Krylov, *Chem. Commun.* **2011**, *47*, 7767 – 7769.
[12] T. J. Johnson, D. Ross, L. E. Locascio, *Anal. Chem.* **2002**, *74*, 45 – 51.
[13] J. R. Pachecoa, K. P. Chen, A. Pacheco-Vega, B. Chen, M. A. Hayes, *Phys. Lett. A* **2008**, *372*, 1001 – 1008.
[14] S. M. Krylova, V. Okhonin, S. N. Krylov, *J. Sep. Sci.* **2009**, *32*, 742 – 756.
[15] V. Okhonin, X. Liu, S. N. Krylov, *Anal. Chem.* **2005**, *77*, 5925 – 5929.
[16] M. Rhee, M. A. Burns, *Langmuir* **2008**, *24*, 590 – 601.
[17] Borland Delphi 7, 2nd edition, 2002.

SUPPORTING INFORMATION

Predictive Measure of Quality of Micromixing

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Table of Contents

1. Supporting Materials and Methods

1.1. Materials. The HPLC-purified, fluorescently-labeled 15-mer DNA (5'-Alexa488-GCG GAG CGT GGC AGG), and complimentary 15-nucleotide DNA (5'-CCT GCC ACG CTC CGC) were purchased from IDT DNA Technology Inc. (Coralville, IA, USA) and dissolved in a TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5) to have 100 μM stock solutions that were stored at -20 °C. All other chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada). Uncoated fused-silica capillaries with 75, 50, and 20 μm inner diameters (375 μm outer diameter) were purchased from Polymicro (Phoenix, AZ, USA). The capillary was mounted on a capillary electrophoresis (CE) instrument (P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA), which was equipped with temperature-controlled sample storage and thermal control of the capillary. All solutions were made using deionized water filtered through a 0.22 μm filter (Millipore, Nepean, ON, Canada).

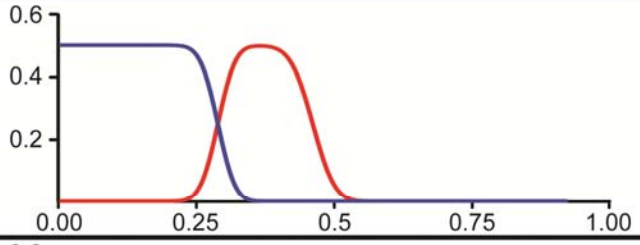
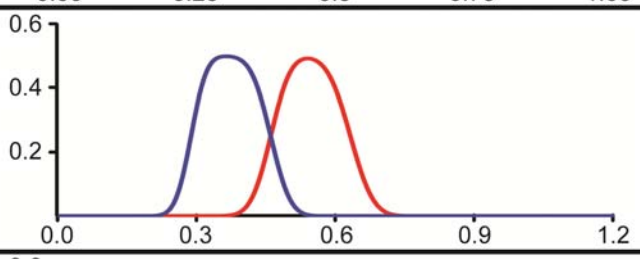
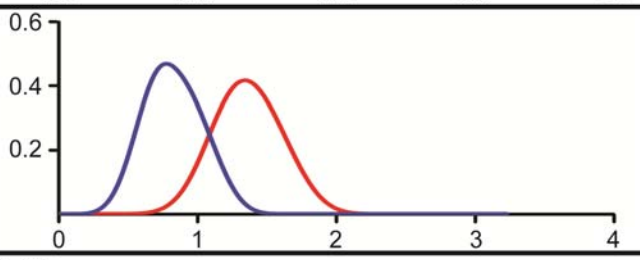
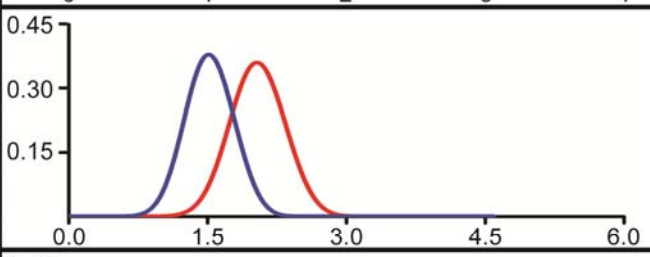
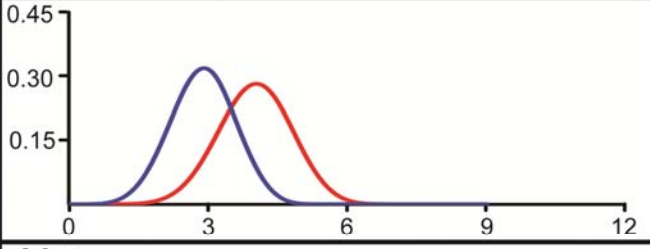
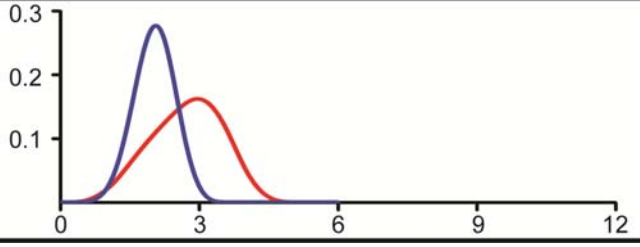
1.2. Instrument modifications. To accurately record pressure profiles, the CE instrument was modified with a commercially-available pressure transducer (MadgeTech PRTrans1000IS Pressure Data Logger). The transducer was attached to the pressure line that feeds the pressure to the capillary inlet. To protect the transducer from excessive pressure, a pressure valve was installed upstream of the transducer. The valve was controlled by a pressure sensor that was set up to close the valve once the pressure was higher than a selected threshold value. The transducer was recording the injection pressure as a function of time and the obtained data was downloaded from the transducer via a USB cable onto a computer using the software provided with the transducer.

1.3. Experimental procedure. The DNA working solutions were prepared separately at a concentration of 500 nM in 100 mM TES buffer pH 7.5. The prepared solutions were injected into a 50-cm capillary, using parameters outlined in **Table S1** below. The injected reactants were incubated in the capillary at room temperature for 1 min to facilitate formation of dsDNA hybrid. The separation in 100 mM TES buffer pH 7.5 was then performed as outlined in **Table S1** below. The separation modes were different to prevent overheating of the capillary and DNA hybrid dissociation.

2. Supporting Results

The obtained electropherograms were analyzed to determine the yield of hybridization reaction. A typical electropherogram with areas highlighted is shown in **Figure S1**. The yield of the hybridization reaction can then be calculated: $Yield = A_{red} / \left(A_{red} + A_{blue} \right)$.

Table S1. Experimental parameters used for TDLFP-based mixing of two reactants and their calculated post-mixing concentration profiles

	Mixing Scenario	Final Reactants Distribution
1	Capillary Diameter: 20 μm Injection Sequence: 1) DNA B: 1 psi × 15 s 2) DNA A: 1 psi × 25 s Separation : 30 kV, 15 min	
2	Capillary Diameter: 20 μm Injection Sequence: 1) DNA B: 1 psi × 15 s 2) DNA A: 1 psi × 15 s 3) Buffer: 1 psi × 25 s Separation: 30 kV, 15 min	
3	Capillary Diameter: 50 μm Injection Sequence: 1) DNA B: 0.5 psi × 15 s 2) DNA A: 0.5 psi × 15 s 3) Buffer: 0.5 psi × 15 s Separation: 1) 10 kV, 10 min 2) 30 kV, 10 min	
4	Capillary Diameter: 50 μm Injection Sequence: 1) DNA B: 0.5 psi × 15 s 2) DNA A: 0.5 psi × 14 s 3) Buffer: 0.5 psi × 35 s Separation: 1) 10 kV, 10 min 2) 30 kV, 10 min	
5	Capillary Diameter: 75 μm Injection Sequence: 1) DNA B: 0.3 psi × 14 s 2) DNA A: 0.3 psi × 14 s 3) Buffer: 0.3 psi × 28 s Separation: 1) 7.5 kV, 10 min 2) 20 kV, 15 min	
6	Capillary Diameter: 75 μm Injection Sequence: 1) DNA B: 0.3 psi × 14 s 2) DNA A: 0.3 psi × 13 s 3) Buffer: 0.3 psi × 35 s Separation: 1) 7.5 kV, 10 min 2) 20 kV, 15 min	

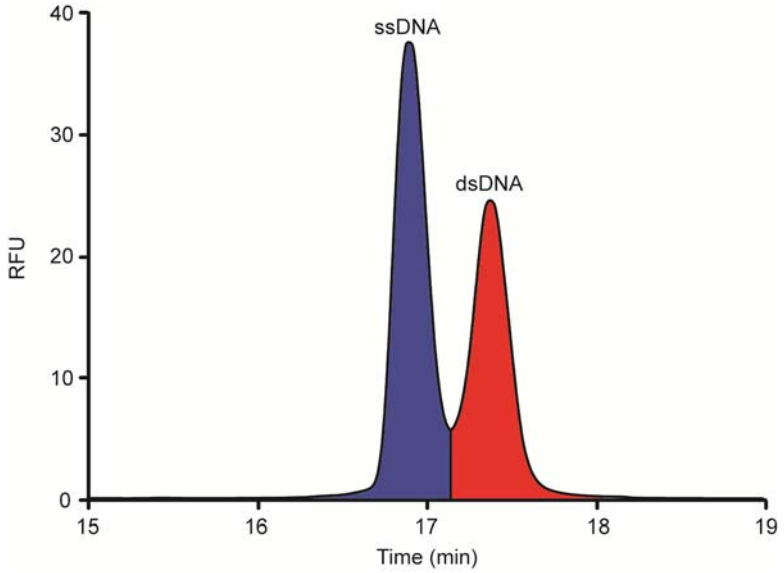


Figure S1. Electrophoretic separation of ssDNA (blue area) from dsDNA (red area).

3. Supporting Mathematics (Properties of *QO*)

Below we present the proof that *QO* satisfies the four conditions described in the main text: (i) $0 \le QO \le 1$, (ii) $QO = 0$ only if there is no a non-zero volume in the reactor where all reactants are present, (iii) $QO = 1$ only if all concentration profiles are similar to each other, i.e. $R_i(\vec{r}) = c_{ij}R_j(\vec{r})$ where constant coefficients c_{ij} do not depend on $\vec{r}$, and (iv) *QO* does not change if an empty volume is added to the system. We also prove the validity of condition (3) in the main text for the linear correlation between QO and product yield.

We assume that all concentrations $R_i(\vec{r})$ are piecewise continuous nonnegative functions in volume *V*. Definition (1) for *QO* presented in the main text can be rewritten as follows:

$$QO(t) = \frac{1}{V}\int_V \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)d\vec{r}\,. \tag{S1}$$

Here we introduce normalized concentrations:

$$R^*_i(\vec{r},t) = \frac{R_i(\vec{r},t)}{\frac{1}{V}\int_V R_i(\vec{r},t)\,d\vec{r}} \quad (i = 1,...,N) \tag{S2}$$

which are also piecewise continuous nonnegative functions in *V*. They obviously satisfy the following relations:

$$\frac{1}{V}\int_V R^*_i(\vec{r},t)\,d\vec{r} = 1 \quad (i = 1,...,N)\,. \tag{S3}$$

Thus, the definition of *QO* is based on the minimum of the normalized concentrations of reactants in any given point of the reactor.

3.1. Proof of the 0 ≤ QO ≤ 1 inequality. Since all $R^*_i \geq 0$, we have:

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) \geq 0 \quad \text{(S4)}$$

and, therefore,

$$QO(t) = \frac{1}{V}\int_V \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) d\vec{r} \geq 0, \quad \text{(S5)}$$

i.e. QO ≥ 0.

On the other hand, it follows from the definition of $\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)$ that

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) \leq R^*_i(\vec{r},t) \quad (i=1,...,N). \quad \text{(S6)}$$

Given (1), (3), and (6), we have:

$$QO(t) = \frac{1}{V}\int_V \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) d\vec{r} \leq \frac{1}{V}\int_V R^*_i(\vec{r},t)\, d\vec{r} = 1 \quad \text{(S7)}$$

and, therefore, $QO \leq 1$.

3.2. Proof of the statement: "*QO* = 0 if and only if there is no a non-zero volume V_0 in the reactor where all reactants are present". Let the condition of $QO = 0$ be true. If there is a volume $V_0 \neq 0$ where all $R_i > 0$, then all $R^*_i > 0$ in V_0 and, therefore, $\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) > 0 \quad \text{in} \quad V_0$ (S8)

Using definition (S1) and inequalities $V \geq V_0$ and $R^*_i \geq 0$, and then taking into account (S8), we have

$$QO(t) = \frac{1}{V}\int_V \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) d\vec{r} \geq \frac{1}{V}\int_{V_0} \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) d\vec{r} > 0, \quad \text{(S9)}$$

i.e. $QO > 0$. This inequality contradicts the condition of $QO = 0$. Thus, our assumption of $V_0 \neq 0$ was false and, therefore, there is no non-zero volume V_0 with all $R_i > 0$ when $QO = 0$.

Now let a volume $V_0 \neq 0$ (with all reactants present) not exist in the reactor. If we have $QO > 0$ in this case, then

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) > 0 \quad \text{in} \quad \text{some} \quad \text{volume} \quad V_0 \neq 0, \quad \text{(S10)}$$

since all R^*_i are piecewise continuous nonnegative functions. As a result, we would have all $R^*_i(\vec{r},t) > 0$ in $V_0 \neq 0$, and, therefore, all $R_i(\vec{r},t) > 0$ in $V_0 \neq 0$. This contradicts the condition of the absence of such a non-zero volume. Thus, the assumption of $QO > 0$ was false and, therefore, $QO = 0$ when there is no non-zero volume V_0 with all reactants present in it.

3.3. Proof of the statement: "*QO* = 1 if and only if all concentration profiles are similar to each other, i.e. $R_i(\vec{r}) = c_{ij}R_j(\vec{r})$ where coefficients c_{ij} do not depend on $\vec{r}$ ". Let the condition of $R_i(\vec{r}) = c_{ij}R_j(\vec{r})$ be true for all possible i and j. Substituting this expression for $R_i(\vec{r})$ into the right hand side of definition (S2) for $R^*_i(\vec{r})$ and taking into account definition (S2) for $R^*_j(\vec{r})$, we have $R^*_i(\vec{r}) = R^*_j(\vec{r})$ for all i and j. Therefore,

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) = R^*_i(\vec{r},t) \quad (i=1,...,N). \quad \text{(S11)}$$

Substituting (S11) into definition (S1) for *QO* and using (S3) we obtain:

$$QO(t)=\frac{1}{V}\int_V \min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)d\vec{r}=\frac{1}{V}\int_V R^*_i(\vec{r},t)\,d\vec{r}=1. \quad \text{(S12)}$$

Thus, $QO = 1$ when $R_i(\vec{r}) = c_{ij}R_j(\vec{r})$.

Now let the condition of $QO = 1$ be true. If we have $R^*_i(\vec{r}) \neq R^*_j(\vec{r})$ for some i, j, and $\vec{r}$, then $R^*_i(\vec{r}) > R^*_j(\vec{r})$ or $R^*_i(\vec{r}) < R^*_j(\vec{r})$. Let us consider for definitiveness the case when the last inequality is satisfied. Such inequality would also hold in a small enough volume $V^*(\vec{r})$ because $R^*_i(\vec{r})$ and $R^*_j(\vec{r})$ are piecewise continuous functions. As a result, we would have

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)\le R^*_j(\vec{r},t) \quad \text{in} \quad V-V^*, \quad \text{(S13)}$$

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)< R^*_j(\vec{r},t) \quad \text{in} \quad V^*. \quad \text{(S14)}$$

Substituting (13) and (14) into definition (1) for QO and using (3) we obtain

$$QO(t)=\frac{1}{V}\left(\int_{V-V^*}\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)d\vec{r}+\int_{V^*}\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)d\vec{r}\right)<$$
$$<\frac{1}{V}\left(\int_{V-V^*}R^*_j(\vec{r},t)\,d\vec{r}+\int_{V^*}R^*_j(\vec{r},t)\,d\vec{r}\right)=\frac{1}{V}\int_V R^*_j(\vec{r},t)\,d\vec{r}=1 \quad \text{(S15)}$$

This result contradicts the condition of $QO = 1$. Thus, the assumption of $R^*_i(\vec{r}) \neq R^*_j(\vec{r})$ was false and, therefore, $R^*_i(\vec{r}) = R^*_j(\vec{r})$ for all i and j when $QO = 1$. Substituting expressions (S2) for $R^*_i(\vec{r})$ and $R^*_j(\vec{r})$ in relation $R^*_i(\vec{r}) = R^*_j(\vec{r})$, we finally obtain that

$$R_i(\vec{r},t)=c_{ij}R_j(\vec{r},t) \quad \text{with} \quad c_{ij}=\frac{\int_V R_i(\vec{r},t)\,d\vec{r}}{\int_V R_j(\vec{r},t)\,d\vec{r}}, \quad \text{when} \quad QO(t)=1. \quad \text{(S16)}$$

3.4. Proof of *QO* not changing upon adding empty volume to the reactor. This statement results from the following relations:

$$QO(V+V_E)=\frac{1}{V+V_E}\int_{V+V_E}\min\left(\frac{R_1(\vec{r},t)}{\frac{1}{V+V_E}\int_{V+V_E}R_1(\vec{r},t)\,d\vec{r}},...,\frac{R_N(\vec{r},t)}{\frac{1}{V+V_E}\int_{V+V_E}R_N(\vec{r},t)\,d\vec{r}}\right)d\vec{r}=$$
$$=\frac{1}{V+V_E}\int_{V+V_E}\frac{1}{\frac{V}{V+V_E}}\min\left(\frac{R_1(\vec{r},t)}{\frac{1}{V}\int_V R_1(\vec{r},t)\,d\vec{r}},...,\frac{R_N(\vec{r},t)}{\frac{1}{V}\int_V R_N(\vec{r},t)\,d\vec{r}}\right)d\vec{r}=QO(V) \quad \text{(S17)}$$

where V_E is an empty volume. In (S17), we took into account that V and V_E do not depend on $\vec{r}$ and used the following relation

$$\int_{V+V_E}R_i(\vec{r},t)\,d\vec{r}=\int_V R_i(\vec{r},t)\,d\vec{r} \quad (i=1,...,N) \quad \text{(S18)}$$

which is valid for any empty volume V_E.

3.5. Proof of *QO* being determined by the concentration of a reactant in deficiency in every point if the total amounts of reactants are similar. The amount A_i of i-th reactant in the reactor is determined as follows:

$$A_i = \int_V R_i(\vec{r},t)\,d\vec{r} \quad (i = 1,...,N)\,. \tag{S19}$$

Using (S19), we can rewrite definition (S2) of the normalized concentration in the form

$$R^*_i(\vec{r},t) = \frac{VR_i(\vec{r},t)}{A_i} \quad (i = 1,...,N)\,, \tag{S20}$$

As a result, the ratio of any two normalized concentrations, R^*_i and R^*_j, is determined by

$$\frac{R^*_i}{R^*_j} = \frac{A_j}{A_i}\frac{R_i}{R_j} \quad (i, j = 1,...,N)\,. \tag{S21}$$

Let us consider the case when all A_i are of the same order of magnitude (i.e. $A_i \sim A_j$ for any possible i and j) and, therefore,

$$\frac{A_j}{A_i} \sim 1 \quad (i = 1,...,N;\ j = 1,...,N)\,. \tag{S22}$$

Relations (S20) –(S22) allow one to approximately calculate *QO* by replacing the exact value of $\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)$ in each point with the normalized concentration of reactant in deficiency in that point. Indeed, if d is the number of a reactant in deficiency in a certain point in the reactor, then we have the following relations between the concentrations in this point: $R_d/R_m << 1$ for some values of $m \neq d$ and $R_d/R_k \sim 1$ for some other values of $k \neq d$ and $k \neq m$. One of the index sets $\{m\}$ and $\{k\}$ can be empty (but not both of them). Using (S21) and (S22), we obtain $R^*_d/R^*_m << 1$ and $R^*_d/R^*_k \sim 1$ for the same values of m and k. Therefore, $\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)$ can be equal only to R^*_d or to R^*_k at some specific value of k (but not to R^*_m at any value of m). As a result, $\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right)$ still can be estimated as R^*_d since $R^*_k \sim R^*_d$ for all values of k from $\{k\}$, and we can approximately calculate *QO* by substituting in (S1) the following expression:

$$\min\left(R^*_1(\vec{r},t),...,R^*_N(\vec{r},t)\right) \approx VR_d(\vec{r},t)/A_d \approx VR_d(\vec{r},t)/A\,, \tag{S23}$$

where R_d is the concentration of the reactant which is in deficiency in point $\vec{r}$, A_d is the total amount of that reactant in the reactor. Values of index d in (S23) can be different in different points of the reactor. However, values of A_d corresponding to all possible values of d have the same order of magnitude according to assumption (S22). This fact allows us to approximately replace A_d with an amount A of one of the reactants in the second relation in (S23). Obviously, the choice of such a reactant cannot significantly affect an estimate (S23). Substituting (S23) into (S1) we finally obtain that

$$QO(t) \approx \frac{1}{A}\int_V R_d(\vec{r},t)\,d\vec{r}\,. \tag{S24}$$

3.6. Proof of condition (3) in the main text being satisfactory for linear correlation between *QO* and the product yield to hold. Condition (3) in the main text can be rewritten in the form

$$\frac{1}{V}\int_V \frac{d\vec{r}}{K_{\text{eq}}R_{\text{excess}}(\vec{r},t)} << 1 . \tag{S25}$$

Since $K_{\text{eq}}R_{\text{excess}}(\vec{r},t) > 0$, inequality (S25) can hold only if $1\Big/\left(K_{\text{eq}}R_{\text{excess}}(\vec{r},t)\right) << 1$ in the most of the volume *V*. Indeed, if

$$\frac{1}{K_{\text{eq}}R_{\text{excess}}(\vec{r},t)} << 1 \quad \text{in} \quad V - V_{\text{m}} \quad \text{and} \quad \frac{1}{K_{\text{eq}}R_{\text{excess}}(\vec{r},t)} \geq 1 \quad \text{in} \quad V_{\text{m}} \tag{S26}$$

then

$$1 >> \frac{1}{V}\int_V \frac{d\vec{r}}{K_{\text{eq}}R_{\text{excess}}(\vec{r},t)} \geq \frac{1}{V}\int_{V_{\text{m}}} \frac{d\vec{r}}{K_{\text{eq}}R_{\text{excess}}(\vec{r},t)} \geq \frac{V_{\text{m}}}{V} . \tag{S27}$$

It follows from (S27) that $V_{\text{m}} << V$ and therefore inequality $1\Big/\left(K_{\text{eq}}R_{\text{excess}}(\vec{r},t)\right) << 1$ is not valid only in a very small part V_{m} of the total volume *V*. The equilibrium constant K_{eq} is defined by

$$K_{\text{eq}} = \frac{P}{R_{\text{excess}}R_d} , \tag{S28}$$

Were *P* and R_{excess} are concentrations of the product and the reactant in excess in any given point of the reactor, R_d, is the concentration of the second reactant in the same point. Relation (S28) holds after the equilibrium is achieved. Substituting (S28) into the first inequality (S26), we have $R_d << P$ in $V - V_{\text{m}} \approx V$. Thus, the reaction proceeds to completion in the largest part of the volume, where most of the product is formed.

Obviously, the local yield of the product in any given point is determined by the initial concentration of the reactant that is in deficiency in this point. Therefore, the total relative yield of the product is determined by the integral of this concentration divided by the initial amount of the labeled reactant. Such a ratio also approximately coincides with *QO* (see (S24)) if the labeled reactant amount was used as *A* in (S24). In this case, one may expect the relative yield of the product to be approximately proportional to the quantitative overlap *QO* since the reaction proceeds to completion when condition (3) in the main text is satisfied.