ChemComm

View Online
Dynamic Article Links

Cite this: *Chem. Commun.*, 2011, **47**, 7767–7769

www.rsc.org/chemcomm

COMMUNICATION

Predictive measure of quality of micromixing†

Alexander P. Petrov, Bryan J. Dodgson, Leonid T. Cherney and Sergey N. Krylov*

Received 23rd April 2011, Accepted 26th May 2011
DOI: 10.1039/c1cc12371h

We introduce a predictive measure of micromixing termed quantitative overlap (*QO*). *QO* depends on the distribution of reactants throughout the reactor and can be calculated by solving equations of diffusion. We used a bimolecular reaction and a capillary microreactor to experimentally prove that *QO* is proportional to the product yield.

Reaction parameters, such as reaction rate and product yield, depend on the quality of reactants' mixing; therefore, the understanding and optimization of reactions require *predictive* quantitative measures of the quality of mixing, which can predict the reaction parameters. In macroreactors, mixing by mechanical agitation (macromixing) is used to randomly break solutions into microvolumes and, thus, aid the final mixing of reactants by diffusion (micromixing). The random nature of macromixing usually makes the entire process of mixing in macroreactors stochastic. Therefore, stochastic approaches are applicable to characterizing the quality of mixing in macroreactors. A number of such approaches have been developed and extensively reviewed.[1,2] A classical example is the Danckwerts approach, which introduces the intensity of segregation, a predictive stochastic measure of mixing, linearly related to the reaction rate.[3] Microreactors are an attractive media for chemical synthesis[4–14] and analysis,[15,16] which have become practical due to technological advances in their manufacturing. Technical difficulties of mechanical agitation in small volumes make macromixing in microreactors cumbersome.[17] On the other hand, micromixing in such reactors may be sufficiently fast, which, in turn, makes macromixing unnecessary.[18,19] Micromixing is not a random process in the scale of the microreactor—the deterministic nature of diffusion leads to well-defined non-random distributions of the reactants throughout the microreactor's volume.[17,20] As a result, the predictive stochastic measures of mixing developed for macromixing are not applicable to micromixing. The quality of micromixing in microreactors was addressed in significantly fewer works. A standard statistical function, coefficient of variation, and some empirical functions were used as quantitative measures of micromixing.[21,22] However, these measures have never been shown to predict any reaction parameter, *e.g.* the rate or product yield of the reaction. Here we introduce the first predictive quantitative measure of micromixing, Quantitative Overlap (*QO*), and demonstrate its predictive ability. In this proof-of-principle work, *QO* was experimentally examined for a bimolecular reaction conducted in a capillary microreactor with discontinuous mixing. This theoretically calculated measure turned out to be proportional to the experimentally determined relative product yield. Due to the generic nature of *QO*, it can be used to characterize micromixing for different reactions in various microreactors, with some restrictions specified below. One of the potential practical applications of *QO* is obvious: the theoretical optimization of microreactors for maximized product yield. Other applications are still to be identified. We also foresee that new quantitative characteristics of micromixing could be introduced to predict reaction parameters other than product yield.

For a quantitative measure to be suitable for comparing efficiencies of micromixing in different systems (reactions and reactors), it should change within the same interval (for example, between 0 and 1) regardless of the system specifics. To correlate with product yield, the parameter has to be maximum for similar distributions of the reactant throughout the reactor and minimum when there is no non-zero volume in which all reactants are present. The definition of the measure's behavior between these two extremes can vary; therefore, different measures can be introduced to be predictive of the product yield in different systems. We present one such parameter, named quantitative overlap (*QO*), that is defined for a general case of N reactants, $R_1,\ldots, R_N$, in the following way:

$$QO(t) = \frac{1}{V}\int_V \min\left(\frac{R_1(\vec{r},t)}{\frac{1}{V}\int_V R_1(\vec{r},t)\mathrm{d}\vec{r}},\ldots,\frac{R_N(\vec{r},t)}{\frac{1}{V}\int_V R_N(\vec{r},t)\mathrm{d}\vec{r}}\right)\mathrm{d}\vec{r} \quad (1)$$

Here $R_1,\ldots, R_N$ are concentrations of the corresponding reactants, V is the volume of the reactor, $\vec{r}$ is a vector of the spatial coordinate, and t is time. $1/V\int$ designates an operation of finding the spatial average of a function over volume V of the reactor. The terms in the brackets are normalized concentrations of reactants (see ESI†). The "min" function in (1) denotes a minimum calculated from a set of the arguments separated by the commas. Relation (1) depends on the number of the reactants rather than on the order of the reaction. *QO* is solely defined by a spatial distribution of reactants throughout the reactor and can change with time whenever the distribution

Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario, Canada. E-mail: skrylov@yorku.ca; Fax: +1 416-736-5936

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc12371h

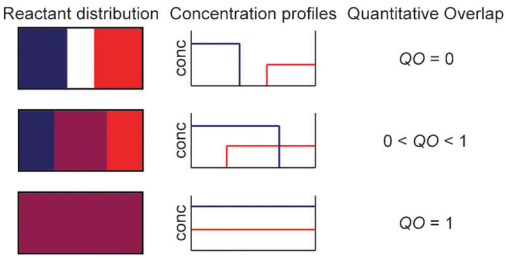


Fig. 1 Illustration of changes in QO as defined by eqn (1) with changing reactant distribution through the reactor.

changes with time. It can be strictly proven that QO, defined by eqn (1), satisfies the following: (i) $0 \leq QO \leq 1$, (ii) $QO = 0$ if and only if there is no non-zero volume in the reactor in which all reactants are present, (iii) $QO = 1$ 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 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. Detailed proofs of these properties of QO are given in the ESI.† For example, if all concentrations are similar to each other then values of all arguments of the "min" function in (1) are the same in each point. This allows the "min" function to be replaced with a value of the first argument. Then, integration in (1) leads to $QO = 1$. Fig. 1 illustrates the change in QO for the changing distribution of 2 reactants in a quasi-1-dimensional reactor, including the two extreme cases of $QO = 1$ and $QO = 0$.

QO can be calculated by solving equations of diffusion (or another process driving micromixing, *e.g.* differential mobility). The solutions will be functions $R_i(\vec{r})$ defined by initial distributions of the reactants and the reactor's geometry. These functions are then used in eqn (1) to calculate QO.

To examine whether or not QO correlates with the product yield, we used a system, in which the reaction and diffusion are uncoupled due to different characteristic times of diffusion and reaction. The reactor was a capillary in which reactants were mixed by transverse diffusion of laminar flow profiles (TDLFP).[15,20,23] Due to a very high ratio between the length and the diameter of such a reactor, transverse diffusion establishes concentration profiles of the reactants much faster than longitudinal diffusion. If the characteristic time of reaction is intermediate to those of transverse and longitudinal diffusions, then the reaction and diffusion are uncoupled. Diffusion in the longitudinal direction can be neglected in the time scale of the reaction, and the reaction can be neglected in the time scale of diffusion in the transverse direction. This allowed us to avoid working in meso-scales.[24]

Fig. S1 in ESI† explains the concept of mixing reactants in a capillary by TDLFP. The solutions of reactants are injected sequentially into a capillary from one end by pressure pulses. For sufficiently short times and high velocities of injection the Peclet number characterizing the injection process is high and fluid profiles of different reactants have shapes of interpenetrating parabolas. The reactants are then mixed by transverse diffusion, eliminating concentration gradients in this direction. Concentration gradients in the longitudinal direction remain since longitudinal diffusion takes much longer to eliminate such gradients. As a result, mixing by TDLFP

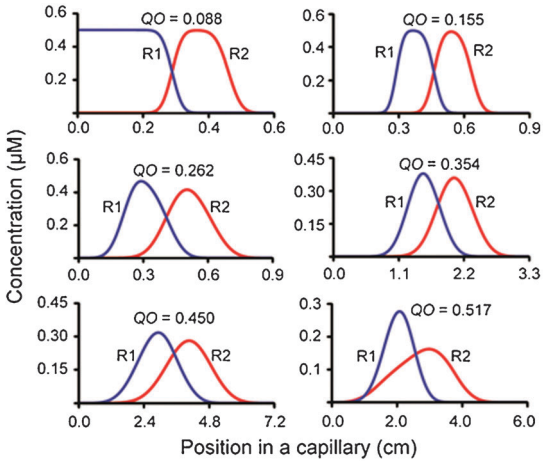


Fig. 2 Calculated concentration profiles of two reactants, R_1 and R_2, after mixing inside a capillary by TDLFP. The corresponding values of QO are shown inside the panels. Capillary diameters, as well as reactant orders, pressures, and times used for the calculations (and experimental injections) are shown in Table S1, ESI.†

establishes stable concentration profiles of reactants along the capillary length. We used these concentration profiles to calculate QO, which we then tested for its correlation to the reaction product yield.

The reaction we considered was hybridization of 2 complementary single-strand DNA molecules, R_1 and R_2. The characteristic time of this reaction, τ_{react}, satisfies the following condition:

$$\tau_{trans} \ll \tau_{react} \ll \tau_{longit}, \quad \tau_{trans} \equiv r^2/D, \quad \tau_{longit} \equiv L^2/D \qquad (2)$$

Here, τ_{trans} and τ_{longit} are characteristic times of diffusion in transverse and longitudinal directions, r is the inner radius of the capillary, L is the length of the injected reactant plugs, and D is the diffusion coefficient. For example, $\tau_{trans} = 6$ s and $\tau_{longit} = 10^6$ s for typical parameters of $r = 25$ μm, $L = 1$ cm, and $D = 10^{-6}$ cm^2 s^{-1}. Different degrees of TDLFP-based mixing were achieved by varying: (i) inner capillary diameter, (ii) pressure used for injection of R_1 and R_2, and (iii) duration of pressure pulses. The after-mixing profiles of linear concentrations, $R_1(x)$ and $R_2(x)$, where x is the distance from the capillary inlet, were calculated for 6 sets of experimental parameters, using an approach described elsewhere[25] and are shown in Fig. 2. The values of QO were calculated using these profiles and eqn (1) and are also shown in Fig. 2. The reactor's volume was defined as a volume where at least one reactant is present. The next goal was to find product yields for different degrees of mixing.

To determine the yield of the hybridization reaction product, P, we fluorescently labeled reactant R_1 so that P was also labeled. After TDLFP-based mixing of R_1 and R_2, the reaction was allowed to proceed to completion, and P was separated from the unreacted R_1 by capillary electrophoresis (see Fig. S2 in ESI†). Peak areas in capillary electrophoresis are proportional to amounts of corresponding analytes. Therefore, the yield of P relative to the amount of injected R_1 was calculated as $A_P/(A_P + A_{R1})$, where A_P and A_{R1} are peak areas corresponding to P and unreacted R_1, respectively. The product yield was determined for all of the mixing

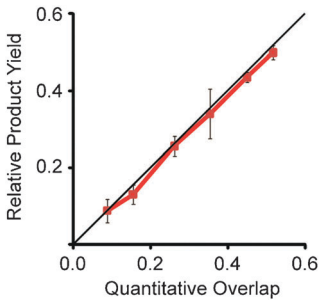


Fig. 3 Dependence of product yield on *QO*. See the text for details.

scenarios shown in Fig. 2. The experiments were done in triplicates. By plotting the experimentally-determined relative product yield *versus* the theoretically calculated *QO*, we found that, remarkably, the two parameters linearly correlate, with an intercept very close to 0 and a slope of approximately 1 (Fig. 3).

This correlation between *QO* and the product yield is not circumstantial. *QO* is based on the minimum of the normalized reactant concentrations in any given point of the reactor (see eqn (1)). If the amounts of all reactants in the reactor (defined by denominators in eqn (1)) are similar, then this minimum identifies the reactant in deficiency in every point, and *QO* can be approximately calculated as an integral of this reactant concentration divided by A, where A is the amount of one of the reactants. The choice of such a reactant cannot significantly affect *QO* since all the reactant amounts are similar (see ESI†). On the other hand, the local product yield cannot exceed the amount of the reactant in deficiency in every point. As a result, the ratio of total product yield to the amount A (*i.e.* relative yield) should be approximately proportional to *QO* if the reaction proceeds to completion almost everywhere in the reactor.

For a bimolecular reaction, the proportionality of the relative product yield and *QO* should stand as long as the following inequality is satisfied (see ESI†):

$$\frac{1}{V}\int_V \frac{\mathrm{d}\vec{r}}{R_{\mathrm{excess}}(\vec{r},t)} \ll K_{\mathrm{eq}} \qquad (3)$$

Here, $R_{\mathrm{excess}}(\vec{r}, t)$ is a spatial equilibrium concentration of a reactant in excess and K_{eq} is the equilibrium thermodynamic constant of the reaction. Satisfying this condition guarantees that most of the product is formed in the parts of the reactor where the reaction proceeds to completion. While there may be parts of the reactor where the reaction does not proceed to completion, only an insignificant fraction of the product could be produced in such parts. Therefore, these parts of the reactor will not significantly distort the correlation between *QO* and the relative product yield.

It should be noted that the choice of the experimental example, a capillary with sequentially injected reactants, is only a matter of convenience. *QO* is applicable to different geometries of microreactors and different scenarios of micromixing. It is applicable, in particular, to continuous-flow microreactors, which are widely used by synthetic chemists.[4–14]

We introduce *QO*, the first predictive quantitative measure, to characterize the degree of micromixing. We experimentally prove that *QO* is predictive of relative product yield by using an example of a bimolecular reaction in a capillary microreactor. The generality of the parameter makes it applicable to different types of reactions and reactors as long as micromixing is a sole means of mixing. *QO* can be used to optimize micromixing for maximum product yield by simply maximizing a single easily calculated parameter (a goal that has, so far, remained very difficult to achieve). Other predictive quantitative measures of micromixing can be designed to serve varying needs. Moreover, being a general mathematical parameter, *QO* can be used in the general field of spatial statistics (population ecology, disease propagation, forestation, geostatistics, *etc.*).

We thank Natural Sciences and Engineering Research Council of Canada for financial support and Dr Victor Okhonin for his valuable suggestions.

Notes and references

1 J. M. Ottino, *The Kinematics of Mixing: Stretching, Chaos, and Transport (Cambridge Texts in Applied Mathematics)*, Cambridge University Press, Cambridge, 1989.
2 K. Ogawa, *Chemical Engineering: A New Perspective*, Elsevier, Amsterdam, 2007, pp. 21–80.
3 P. V. Danckwerts, *Appl. Sci. Res.*, 1952, **3**, 279–296.
4 Z. N. Pierre, C. R. Field and A. Scheeline, *Anal. Chem.*, 2009, **81**, 8496–8502.
5 J. P. McMullen and K. F. Jensen, *Annu. Rev. Anal. Chem.*, 2010, **3**, 19–42.
6 E. Comer and M. G. Organ, *J. Am. Chem. Soc.*, 2005, **127**, 8160–8167.
7 S. Mozharov, A. Nordon, D. Littlejohn, C. Wiles, P. Watts, P. Dallin and J. M. Girkin, *J. Am. Chem. Soc.*, 2011, **133**, 3601–3608.
8 A. Polyzos, M. O'Brien, T. P. Petersen, I. R. Baxendale and S. V. Ley, *Angew. Chem., Int. Ed.*, 2011, **50**, 1190–1193.
9 J. Wegner, S. Ceylan, C. Friese and A. Kirschning, *Eur. J. Org. Chem.*, 2010, 4372–4375.
10 H. Kawanami, K. Matsushima, M. Sato and Y. Ikushima, *Angew. Chem., Int. Ed.*, 2007, **46**, 5129–5132.
11 M. Rasheed and T. Wirth, *Angew. Chem., Int. Ed.*, 2011, **50**, 357–358.
12 H. Song, J. Tice and R. Ismagilov, *Angew. Chem., Int. Ed.*, 2003, **42**, 767–772.
13 R. Schenk, V. Hessel, C. Hofmann, J. Kiss, H. Lowe and A. Ziogas, *Chem. Eng. J.*, 2004, **101**, 421–429.
14 C. Wiles and P. Watts, *Eur. J. Org. Chem.*, 2008, 1655–1671.
15 S. M. Krylova, V. Okhonin, C. J. Evenhuis and S. N. Krylov, *TrAC, Trends Anal. Chem.*, 2009, **28**, 987–1010.
16 J. West, M. Becker, S. Tombrink and A. Manz, *Anal. Chem.*, 2008, **80**, 4403–4419.
17 S. Hardt and F. Schönfeld, *Microfluidic Technologies for Miniaturized Analysis Systems*, Springer Science + Business Media, New York, 2007.
18 H. Okamotoa, T. Ushijima and O. Kitoh, *Chem. Eng. J.*, 2004, **101**, 57–63.
19 T. Kirner, J. Albert, M. Günther, G. Mayer, K. Reinhäckel and J. M. Köhler, *Chem. Eng. J.*, 2004, **101**, 65–74.
20 S. M. Krylova, V. Okhonin and S. N. Krylov, *J. Sep. Sci.*, 2009, **32**, 742–756.
21 J. Aubin, D. F. Fletcher, J. Bertrand and C. Xuereb, *Chem. Eng. Technol.*, 2003, **26**, 1262–1270.
22 T. J. Johnson, D. Ross and L. E. Locascio, *Anal. Chem.*, 2002, **74**, 45–51.
23 E. Wong, V. Okhonin, M. V. Berezovski, T. Nozaki, H. Waldmann, K. Alexandrov and S. N. Krylov, *J. Am. Chem. Soc.*, 2008, **130**, 11862–11863.
24 J. Li, W. Ge, W. Wang and M. Kwauk, *Particuology*, 2010, **8**, 634–639.
25 D. Yunusov, M. So, S. Shayan, V. Okhonin, M. U. Musheev, M. V. Berezovski and S. N. Krylov, *Anal. Chim. Acta*, 2009, **631**, 102–107.

SUPPORTING INFORMATION

Predictive Measure of Quality of Micromixing

Alexander P. Petrov, Bryan Dodgson, Leonid T. Cherney, and Sergey N. Krylov

Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario, M3J 1P3, Canada

Table of Contents

Page number

1. Supporting Materials and Methods …… S2
   - 1.1. Materials…….. S2
   - 1.2. Instrument modifications…..…….. S2
   - 1.3. Experimental procedure…….. S2
2. Supporting Results……..…… S2
   - Table S1……..……..……. S3
   - Figure S1…….. S4
   - Figure S2…….. S4
3. Supporting Mathematics (Properties of *QO*)…… S4
   - 3.1. Proof of the $0 \leq QO \leq 1$ inequality….……..…….. S5
   - 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"…….. S5
   - 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 $\vec{r}$ "….....……..…….. S6
   - 3.4. Proof of *QO* not changing upon adding empty volume to the reactor…….. S7
   - 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 …..... S7
   - 3.6. Proof of condition (3) in the main text being satisfactory for linear correlation between *QO* and the product yield to hold……... S8

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).

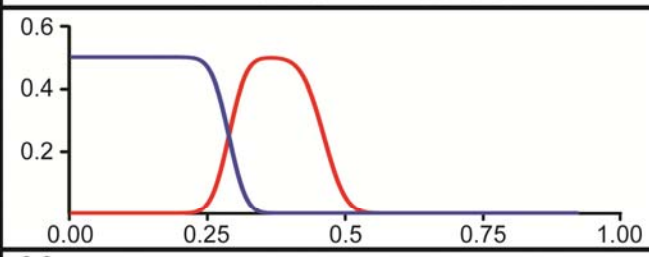
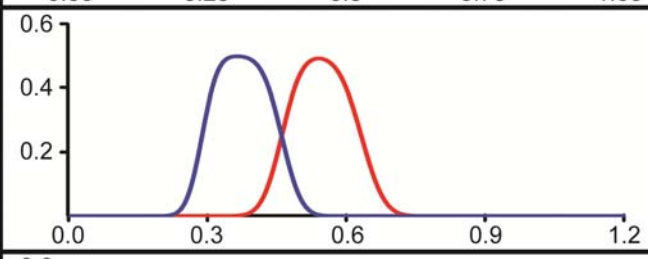
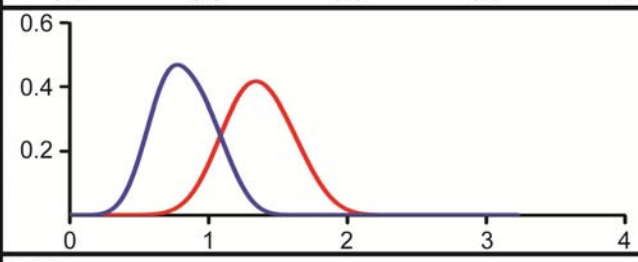
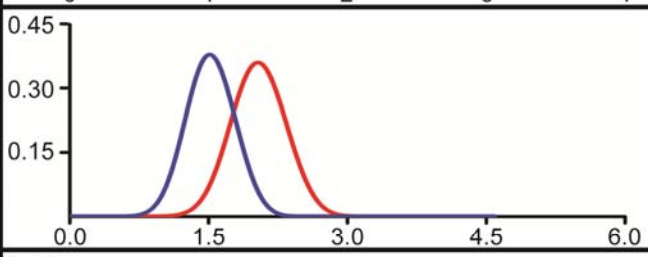
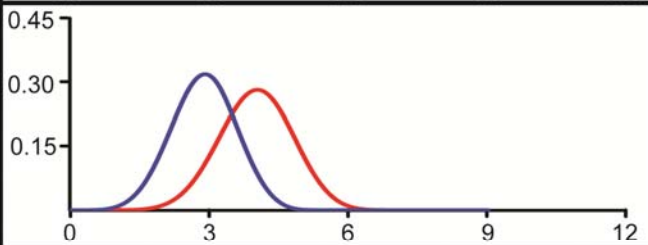
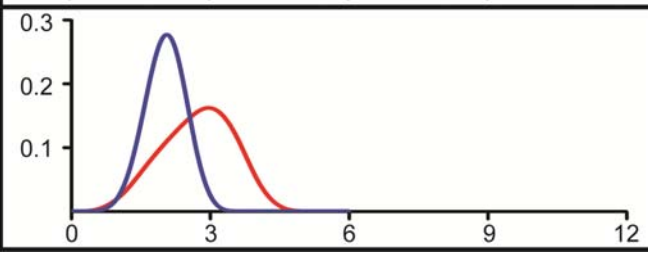
1.2. Instrument modifications. To accurately record pressure profiles, the CE instrument was modified with a commercially-available pressure transducer (MadgeTech PRTrans1000IS Pressure Data Logger). The transducer was attached to the pressure line that feeds the pressure to the capillary inlet. To protect 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	

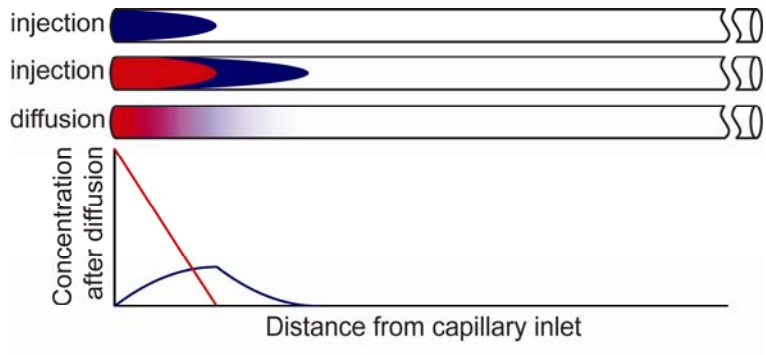


Figure S1. Schematic representation of in-capillary mixing of two solutions, blue and red, by TDLFP. The top panel shows the two steps in mixing: sequential injection of the solutions and their transverse diffusion. The bottom panel shows the concentration distribution after this mixing.

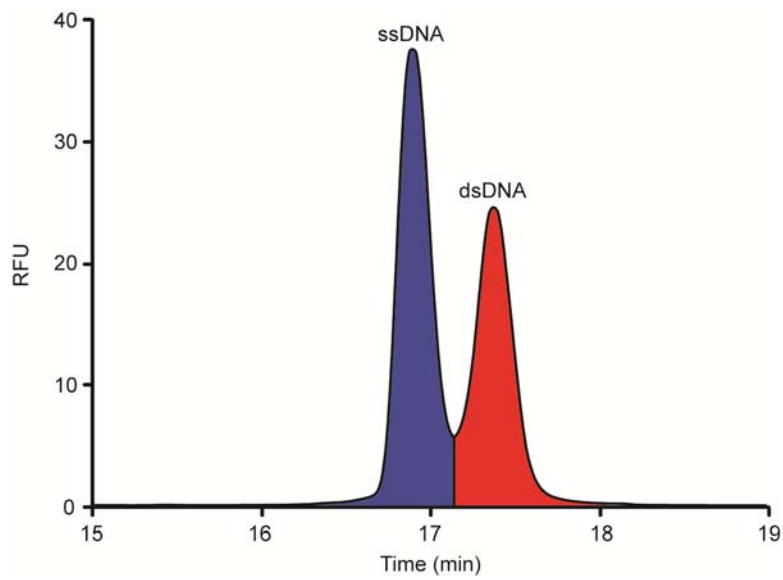


Figure S2. Electrophoretic separation of ssDNA (blue area) from dsDNA (red area).

3. Supporting Mathematics (Properties of *QO*)

Below we present the proof that *QO* satisfies the four conditions described in the main text: (i) $0 \leq QO \leq 1$, (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:

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

and, therefore,

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

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

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

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

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

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

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

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

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

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,

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

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

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

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

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

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

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

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

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

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

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

$$QO(V+V_{\mathrm{E}}) = \frac{1}{V+V_{\mathrm{E}}} \int\limits_{V+V_{\mathrm{E}}} \min\left(\frac{R_1(\vec{r},t)}{\frac{1}{V+V_{\mathrm{E}}} \int\limits_{V+V_{\mathrm{E}}} R_1(\vec{r},t)\,d\vec{r}}, \ldots, \frac{R_N(\vec{r},t)}{\frac{1}{V+V_{\mathrm{E}}} \int\limits_{V+V_{\mathrm{E}}} R_N(\vec{r},t)\,d\vec{r}} \right) d\vec{r} =$$

$$= \frac{1}{V+V_{\mathrm{E}}} \int\limits_{V+V_{\mathrm{E}}} \frac{1}{\frac{V}{V+V_{\mathrm{E}}}} \min\left(\frac{R_1(\vec{r},t)}{\frac{1}{V}\int\limits_{V} R_1(\vec{r},t)\,d\vec{r}}, \ldots, \frac{R_N(\vec{r},t)}{\frac{1}{V}\int\limits_{V} R_N(\vec{r},t)\,d\vec{r}} \right) d\vec{r} = QO(V) \quad , \tag{S17}$$

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

$$\int\limits_{V+V_{\mathrm{E}}} R_i(\vec{r},t)\,d\vec{r} = \int\limits_{V} R_i(\vec{r},t)\,d\vec{r} \quad (i=1,\ldots,N) \tag{S18}$$

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:

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

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

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

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

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

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,

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

Relations (S20) –(S22) allow one to approximately calculate *QO* by replacing the exact value of $\min\left(R^*_1(\vec{r},t),\ldots,R^*_N(\vec{r},t)\right)$ 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 $1/\left(K_{\text{eq}}R_{\text{excess}}(\vec{r},t)\right) << 1$ 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 $1/\left(K_{\text{eq}}R_{\text{excess}}(\vec{r},t)\right) << 1$ 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.