

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*

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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 μ M 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 μ m inner diameters (375 μ m 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 μ m 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: $Yield = A_{red} / (A_{red} + A_{blue})$.

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 \times 15 s 2) DNA A: 1 psi \times 25 s Separation : 30 kV, 15 min	
2	Capillary Diameter: 20 μm Injection Sequence: 1) DNA B: 1 psi \times 15 s 2) DNA A: 1 psi \times 15 s 3) Buffer: 1 psi \times 25 s Separation: 30 kV, 15 min	
3	Capillary Diameter: 50 μm Injection Sequence: 1) DNA B: 0.5 psi \times 15 s 2) DNA A: 0.5 psi \times 15 s 3) Buffer: 0.5 psi \times 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 \times 15 s 2) DNA A: 0.5 psi \times 14 s 3) Buffer: 0.5 psi \times 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 \times 14 s 2) DNA A: 0.3 psi \times 14 s 3) Buffer: 0.3 psi \times 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 \times 14 s 2) DNA A: 0.3 psi \times 13 s 3) Buffer: 0.3 psi \times 35 s Separation: 1) 7.5 kV, 10 min 2) 20 kV, 15 min	

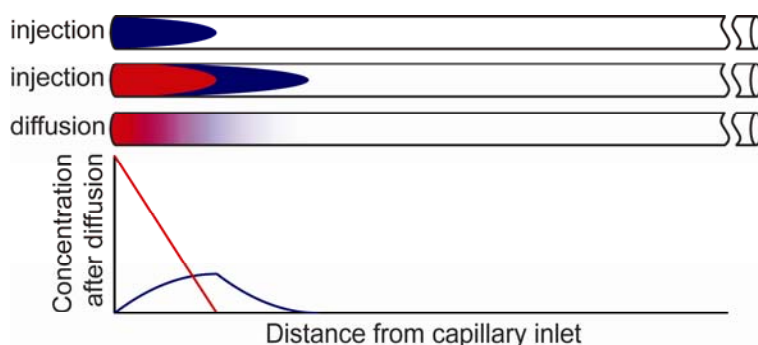


Figure S1. Schematic representation of in-capillary mixing of two solutions, blue and red, by TDLFP. The top panel shows the two steps in mixing: sequential injection of the solutions and their transverse diffusion. The bottom panel shows the concentration distribution after this mixing.

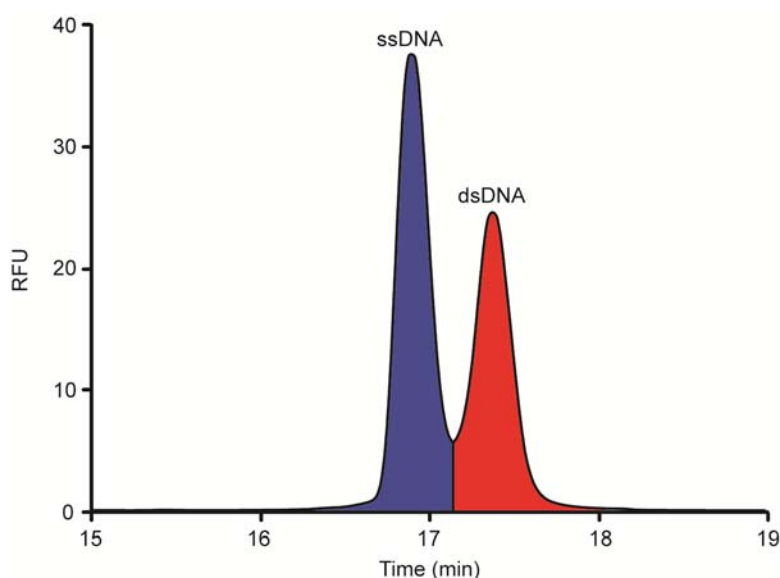


Figure S2. Electrophoretic separation of ssDNA (blue area) from dsDNA (red area).

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. $R_i(\bar{r}) = c_{ij}R_j(\bar{r})$ where constant coefficients c_{ij} do not depend on \bar{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 $R_i(\bar{r})$ are piecewise continuous nonnegative functions in volume V . Definition (1) for QO presented in the main text can be rewritten as follows:

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

Here we introduce normalized concentrations:

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

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

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

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 $0 \leq QO \leq 1$ inequality. Since all $R^*_i \geq 0$, we have:

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

and, therefore,

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

i.e. $QO \geq 0$.

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

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

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

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

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, $\min(R^*_1(\vec{r}, t), \dots, R^*_N(\vec{r}, t)) > 0$ in 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

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

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

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

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,

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

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

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

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

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

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

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

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

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

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

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

$$\begin{aligned}
 QO(V+V_E) &= \frac{1}{V+V_E} \int_{V+V_E} \min \left(\frac{R_1(\vec{r}, t)}{\frac{1}{V+V_E} \int_{V+V_E} R_1(\vec{r}, t) d\vec{r}}, \dots, \frac{R_N(\vec{r}, t)}{\frac{1}{V+V_E} \int_{V+V_E} R_N(\vec{r}, t) d\vec{r}} \right) d\vec{r} = \\
 &= \frac{1}{V+V_E} \int_{V+V_E} \frac{1}{V} \min \left(\frac{R_1(\vec{r}, t)}{\frac{1}{V} \int_V R_1(\vec{r}, t) d\vec{r}}, \dots, \frac{R_N(\vec{r}, t)}{\frac{1}{V} \int_V R_N(\vec{r}, t) d\vec{r}} \right) d\vec{r} = QO(V)
 \end{aligned} \tag{S17}$$

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

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

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:

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

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

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

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

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

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,

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

Relations (S20)–(S22) allow one to approximately calculate QO by replacing the exact value of $\min(R^*_1(\vec{r}, t), \dots, R^*_N(\vec{r}, t))$ 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: $R_d/R_m \ll 1$ 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 $R^*_d/R^*_m \ll 1$ and $R^*_d/R^*_k \sim 1$ for

the same values of m and k . Therefore, $\min(R^*_{1}(\vec{r},t), \dots, R^*_{N}(\vec{r},t))$ 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, $\min(R^*_{1}(\vec{r},t), \dots, R^*_{N}(\vec{r},t))$ 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:

$$\min(R^*_{1}(\vec{r},t), \dots, R^*_{N}(\vec{r},t)) \approx VR_d(\vec{r},t)/A_d \approx VR_d(\vec{r},t)/A, \quad (\text{S23})$$

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

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

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

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

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

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

then

$$1 \gg \frac{1}{V} \int_V \frac{d\vec{r}}{K_{\text{eq}} R_{\text{excess}}(\vec{r},t)} \geq \frac{1}{V} \int_{V_m} \frac{d\vec{r}}{K_{\text{eq}} R_{\text{excess}}(\vec{r},t)} \geq \frac{V_m}{V}. \quad (\text{S27})$$

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

$$K_{\text{eq}} = \frac{P}{R_{\text{excess}} R_d}, \quad (\text{S28})$$

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 $R_d \ll P$ in $V - V_m \approx$

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.