Design of Fast Microfluidic Protein Folding Devices by a Semi-Deterministic Global Optimization Algorithm

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Résumé :

Un nouvel algorithme d'optimisation globale est utilisé pour la conception d'un mélangeur microfluidique. Notre objectif est de modifier la forme de ce dispositif afin de réduire le temps de mélange pour le repliement de protéines.

Abstract :

A new semi-deterministic global optimization algorithm is used for the design of a fast-micro-mixer. Our aim is to reduce the mixing time for protein folding by modifying the device shape.

Mots-clefs :

Shape optimization ; Global optimization ; Microfluidic mixers

1 Introduction

Microfluidic channel systems used in bio-analytical applications are fabricated using technologies derived from microelectronics industry including lithography, wet etching and bonding of substrates. Industrial applications of these techniques concern DNA sequencing, new drug molecules trials, pollution detection in water or food, protein folding ...

Focusing on this last domain, important structural events occur on a microsecond time scale [1]. To study their kinetics, folding reactions must be initiated at even shorter timescales. This for instance using photochemical initiation and changes in temperature pressure or chemical potential, as in salt or chemical denaturant concentration changes [2]. All these technics provide the perturbation of protein conformational equilibrium necessary to initiate folding. In comparison to temperature- and pressure-jump relaxation techniques, folding experiments based on changes in chemical potential, via rapid mixing of protein solutions into and out of chaotrope solvents, are more versatile. The technique is applicable to a wide range of proteins as most unfold reversibly in the presence of chemical denaturants such as urea and guanidine hydrochloride (GdCl) [2]. Further, mixer-based experiments are not limited to proteins near the folding transition state.

Until recently, the main limitation of mixer-based experiments was their inability to access very short timescales. Mixing of chemical species is ultimately limited by the time required for molecular diffusion across a finite length scale, and diffusion time scales as the square of diffusion length. Brody et al. [3] first proposed rapid mixers based on hydrodynamic focusing as a way to address the issue of reducing diffusion lengths under laminar flow conditions while minimizing sample consumption. Hydrodynamic focusing has been used to measure protein and RNA folding [4], with mixing times of a few hundreds of microseconds.

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Our mixer is based on a continuous flow principle by Knight et al. [5] which leverages hydrodynamic focusing on the micron scale to reduce diffusion lengths. This mixing method uses hydrodynamic focusing to form a sub-micron liquid stream of denatured protein solution. As denaturant diffuses away from the stream, individual proteins experience a decreasing local denaturant concentration and start to refold.

This paper discusses specific shape optimization for our new microfluidic mixer [6], in order to reduce its mixing time. In section 2, we introduce our algorithm and a short mathematical background. In section 3, we give the physical and mathematical modelling of our mixer. Section 4 presents the result achieved with our method and compare it to the initial shape mixer.

2 Global optimization method

We want to minimize a functional $J : \Omega \rightarrow \mathbb{R}$ (where Ω is a subset of $\mathbb{R}^n$) with the following hypotheses [7] :

-H1 :$J \in C^1(\Omega, \mathbb{R})$ and $J(x)$ tends to $+\infty$ when $|x|$ tends to $+\infty$.

-H2 : J_m is the minimum of J and there exists $x_m \in \Omega$ such that $J(x_m) = J_m$. In cases where J_m is unknown, we set $J_m = -\infty$ and look for the best solution for a given complexity and computational effort.

The general idea of the Semi Determinsitic Algorithm (SDA) is to improve the efficiency of any particular local deterministic minimization algorithms (gradient, Newton, etc...), by making it global. For sake of simplicity, we will only consider here the following optimal descent algorithm with an output called $D(x_0, I, \epsilon)$:

- **Input :** x_0, I, ϵ
- $x_1 = x_0$

For n going from 1 to I
- Determine $\rho_{opt} = argmin_\rho(J(x_n - \rho \nabla J(x_n)))$ using dichotomy
- $x_{n+1} = x_n - \rho_{opt} \nabla J(x_n)$
- **If** $J(x_{n+1}) < J_m + \epsilon$ **EndFor**

EndFor
- **Output :** $D(x_0, I, \epsilon) = x_{n+1}$

where the inputs $x_0 \in \Omega$, $\epsilon \in \mathbb{R}^+$ and $I \in \mathbb{N}$ are respectively the initial condition, the stopping criterion and the iteration number.

We consider that the minimization problem is solved if and only if the initial condition x_0 lies in the global minimum attraction basin of J. In order to determine such an initial condition, we consider $x_0 = v$ as a new variable in the previous algorithm to be found by the minimization of :

$$h(v) = J(D(v, I, \epsilon)) - J_m \tag{1}$$

To perform the minimization of (1), we then consider the algorithm, with an output called $A_1(v_1, N, I, \epsilon)$, presented in Figure 1-*left*. Where $v_1 \in \Omega$, $(N, I) \in \mathbb{N}^2$ and $\epsilon \in \mathbb{R}^+$.

As this line search minimization algorithm might fail, an external level to the algorithm A_1 is added in order to have a multidimensional search. As previously, we consider $v_1 = w$ as a new variable in A_1 to be found by the minimization of :

$$\tilde{h}(w) = h(A_1(w, N, I, \epsilon)) \tag{2}$$

• **Input :** v_1, N, ϵ
• v_2 chosen randomly
For i going from 1 to N
  • $o_i = D(v_i, \epsilon)$
  • $o_{i+1} = D(v_{i+1}, \epsilon)$
  • **If** $J(o_i) = J(o_{i+1})$ **EndFor**
  • **If** $min\{J(o_k), k = 1, ..., i\} < J_m + \epsilon$ **EndFor**
  • $v_{i+2} = v_{i+1} - h(v_{i+1}) \frac{v_{i+1} - v_i}{J(o_{i+1}) - J(o_i)}$
EndFor
• **Output :** $A_1(v_1, N, \epsilon)$:
$argmin\{J(o_k), k = 1, ..., i\}$

• **Input :** w_1, M, N, ϵ
• w_2 chosen randomly
For i going from 1 to M
  • $p_i = A_1(w_i, \epsilon)$
  • $p_{i+1} = A_1(w_{i+1}, \epsilon)$
  • **If** $J(p_i) = J(p_{i+1})$ **EndFor**
  • **If** $min\{J(p_k), k = 1, ..., i\} < J_m + \epsilon$ **EndFor**
  • $w_{i+2} = w_{i+1} - \tilde{h}(w_{i+1}) \frac{w_{i+1} - w_i}{J(p_{i+1}) - J(p_i)}$
EndFor
• **Output :** $A_2(w_1, M, N, \epsilon)$:
$argmin\{J(p_k), k = 1, ..., i\}$

FIG. 1 – **Left :** Algorithm $A_1(v_1, N, \epsilon)$. **Right :** Algorithm $A_2(w_1, M, N, \epsilon)$.

To perform the minimization of (2), we then consider the two-level algorithm, with an output called $A_2(w_1, M, N, I, \epsilon)$, presented in Figure 1-*right*. Where $w_1 \in \Omega$, $(M, N, I) \in \mathbb{N}^3$ and $\epsilon \in \mathbb{R}^+$. In order to add search directions, the previous construction can be easily pursued recursively.

The choice of the initial condition w_1 in this algorithm contains the only non-deterministic feature of the SDA method. In practice we randomly choose the initial condition $w_1 \in \Omega$ and we consider $(N, M, I) = (5, 5, 10)$. These values give a good compromise between computation complexity and result accuracy. Mathematical background for this approach and validation on academic test cases are available [7, 8].

3 Micro-mixer modelling

We want to optimize a part of our microfluidic shape in order to reduce its mixing time. To solve this problem using the previous presented algorithm we need to derive a mathematical modelling.

3.1 Shape design

The mixer shape considered is a typical three-inlet/single-outlet channel architecture proposed by Knight [5] (see Figure 2-Left). Due to the fact that our model is symmetrical we only study the half of the mixer [6] (see figure 2-Center). Our aim is to optimize the corner shapes. We parameterize the corner regions by cubic splines (see Figure 2-Right). The total number of parameters is 8, 4 for each corner.

In addition, a number of physical limitations of the problem impose constraints on the optimization. The considered Fast-Micro-Mixer is $22\mu m$ long and $10\mu m$ large. The lithography step in fabrication limits the minimum feature size to 1 - 2 μm. We also fix the width of the side channel nozzles to 3 μm and the width of the center channel nozzles to 2 μm to mitigate clogging issues. We constrained the depth of the channels to 10 μm to optimize the fluorescence signal with a confocal system and because we intend to build future devices out of fused silica which is difficult to etch deeper.

Thus, the corresponding search space of the optimization problem is $\Omega = [x_i^{min}, x_i^{max}]_{i=1}^{8}$ where x_i^{min} (resp. x_i^{max}), the minimum (resp. maximum) value of the i^{th} parameter, are fixed by the previous constraints.

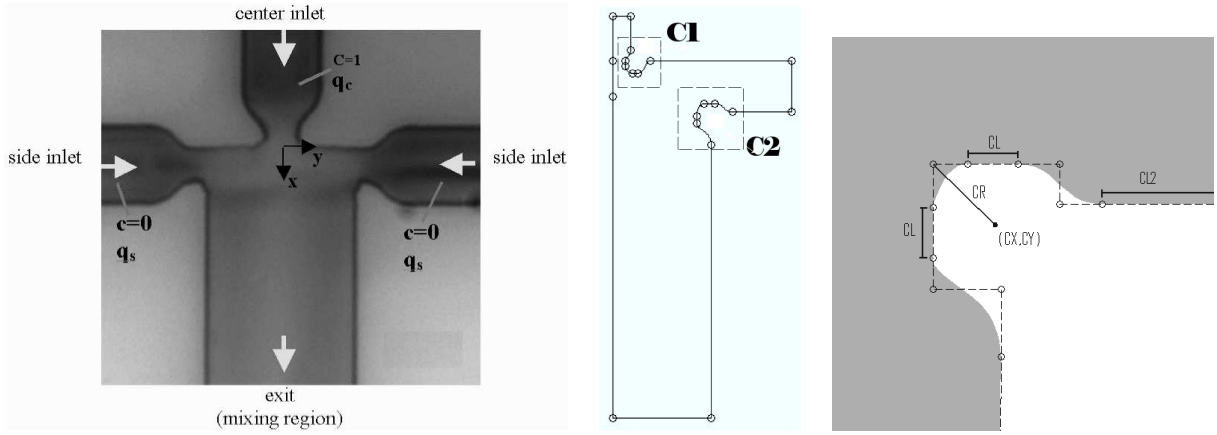

FIG. 2 – **Left :** Typical micro-mixer geometry. q_s and q_c are respectively the side/center injection velocities. c is the denaturant concentration. **center :** Half-mixer Shape parameterization. Corners are denoted by C_1 and C_2. **Right :**Typical parameterization of a corner. Here C_2. We consider 4 parameters : C_x, C_r, C_l, C_{l2}. C_y is fixed.

3.2 State equations

The mixer flow was analyzed using numerical solutions of the full Navier-Stokes fluid flow equations and a convective diffusion equation describing concentration fields c of the guanidine hydrochloride denaturant. Only steady configurations have been considered as we are not interested in the behavior of the device during its transient set up.

These flow simulations were used to explore the guanidine hydrochloride performance of a variety of mixer designs with systematically varied flow and geometric parameters. The model is applied to mixer shape designs described in section 3.1. The basic design consists of a sample stream that enters the mixing region through a center nozzle, focused by two symmetric side channels. We approximate flow at the vertical midplane with two-dimensional (2D) flow simulations [9]. Our aim is to use the lowest complexity possible for the state equation to make the optimization cheap. A posteriori prototyping has shown that this low complexity model was valid as the functioning of the device correspond to what expected from the numerical results with an mixing time error of $\sim 5\%$ [6]. Thus the two considered equations are given by :

$$-\nabla.(\eta(\nabla u + (\nabla u)^\top)) + \rho(u.\nabla)u + \nabla p = 0; \nabla.u = 0 \tag{3}$$

where (u, p) is the flow velocity vector and pressure field, $\rho = 1,013 kg/m^3$ is the density and $\eta = 1 \times 10^{-3} kg/ms$ the dynamic viscosity.

$$\nabla.(-D\nabla c + cu) = 0 \tag{4}$$

where $D = 2 \times 10^{-9} m^2/s$ is the diffusion coefficient.

Finally, the following boundary conditions are assumed : $u = 0$ on shape border, $u = 3.2 \times 10^{-4} m/s$ on side inlets, $u = 3.2 \times 10^{-6} m/s$ on center inlet, $u.t = 0$ on the exit, $u.n = 0$

on the center symmetry line. (t, n) is the local orthonormal reference frame along the boundary. c is prescribed at inlet and normal zero gradient is assumed for all other boundaries. $c = 0$ at side inlet and $c = 1$ at center inlet.

In order to achieve a numerical solution, the Incompressible Navier-Stokes equation non-linear solver solves the equations iteratively. It uses Lagrange P2-P1 elements to stabilize the pressure and to realize the Ladyzhenskaya, Babouska and Brezzi (LBB) stability condition. The convective diffusion equation is solved using a streamline-upwind/Petrov-Galerkin (SUPG) method in order to stabilize the advection. These both stabilization techniques prevent numerical oscillations and instabilities. A Direct Damped Newton method is then used to solve the linear systems leaking from 3- 4 [7].

3.3 Cost Function

The cost function to minimize is the mixing time of the considered Lagrangian fluid particle travelling along the centerline into our microfluidic-mixer with a shape associated to $x_{shape} \in \Omega$. In this paper, we define mixing time as the time required to change the concentration of a typical protein particle from 90% to 30% of the initial value c_0. Then the cost function is given by :

$$J(x_{shape}) = \int_{c_{90}^{x_{shape}}}^{c_{30}^{x_{shape}}} \frac{dy}{u^{x_{shape}}(y).t} \tag{5}$$

Where $c_{90}^{x_{shape}}$ and $c_{30}^{x_{shape}}$ denote respectively the points along the symmetry line where the concentration is at 90% and 30% of c_0.

4 Shape optimization results

We want to optimize the mixing time cost function (5) of the micro-fluidic mixer defined in section 3.1 by controlling its corner shape design. The two-level SDA algorithm $A_2(w_1, M, N, I, \epsilon)$ is used to minimize the cost function, with $w_1 \in \Omega$ fixed and with the following given values : $N = 5$, $M = 5$, $I = 10$, $\epsilon = 1 \times 10^{-4}$. J_m is unknown, as we precise in section 2 we set the cost function infimum at $J_m = -\infty$.

The SDA starts from an initial shape made with 90 degrees corners parameterized with splines to keep the admissible regularity. The mixing time have been decreased from $8\mu s$ for the initial shape to $1.15\mu s$ for the optimized shape (see Figure 3-c). The total number of functional evaluations is $\sim$3600. Each evaluation requires between 20 seconds and one minute on a 3Ghz PC computer. Hence, SDA requires about one day. Convergence histories are given in Figure 3-b. As we can see, SDA has visited several attraction basins before exploring the best element basin, the problem is non-linear, so the use of a global optimization tool is justified. The shape obtained with the SDA method is presented in Figure 3-a. This shape is not intuitive.

5 conclusion

A new semi-deterministic optimization algorithm has been presented and has permitted to optimize a microfluidic shape in order to reduce the mixing time. The obtained geometries have been validated by a posteriori prototyping showing the validity of the approach and the pertinence of the physical modelling based on Navier-Stokes equations and transport-diffusion of ribosome concentration in the flow.

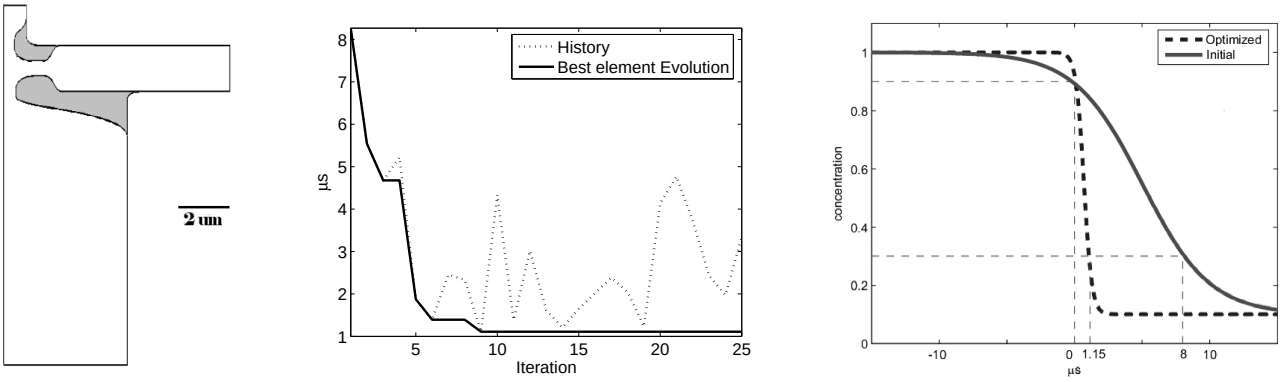

FIG. 3 – **Form left to right : a** : SDA optimized shape superposed over Initial shape. Parts in grey have been removed by the algorithm. **b** : Best element convergence (solid line) and global convergence history (dashed line) vs. iteration. Each iteration corresponds to the best element found after the steepest descent method. **c** : Concentration evolution for the initial and SDA optimized shapes.

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