文章编号 1004-924X(2009)06-1403-06

熵受限在纳流控生物分子分离系统中的应用

LI Zi-rui[1], LIU Gui-rong[1,2], HAN Jongyoon[3], WANG Jian-sheng[1,4], CHEN Yu-zong[1,5]

1. The Singapore-MIT Alliance (SMA), Singapore 117576
2. Department of Mechanical Engineering, National University of Singapore, Singapore 117576
3. Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
4. Department of Physics, National University of Singapore, Singapore 117542
5. Department of Pharmacy, National University of Singapore, Singapore 117543

摘要:提出了生物分子在深度周期性变化纳米流控通道中输运的理论模型。该系统利用不同大小的非各向同性粒子处在两个平面组成的狭小空间时转动自由度受限制程度(熵受限)的不同来实现带电粒子的分离。基于一维简化模型,建立了有效迁移率与通道尺寸、分子大小以及外电场强度的关系的解析解,用于表明这些因素如何决定分子分离的效果。算例表明对于 50, 150 和 300 bp 的 DNA 片段,在低电场强度下,迁移率误差值在 5%以下。该简化模型可用于分析和优化实际的纳滤分离系统,而无需做复杂的数值模拟,省去了大量的物理实验过程。

关 键 词:DNA;电泳;分离技术;纳流系统;熵受限;生物分子分离;生物芯片

中图分类号:Q78;Q829 **文献标识码**:A

Role of configurational entropy in molecular sieving through nanofilter arrays

LI Zi-rui[1], LIU Gui-rong[1,2], HAN Jongyoon[3], WANG Jian-sheng[1,4], CHEN Yu-zong[1,5]

(1. *The Singapore-MIT Alliance (SMA), Singapore* 117576;
2. *Department of Mechanical Engineering, National University of Singapore, Singapore* 117576;
3. *Massachusetts Institute of Technology, Cambridge, Massachusetts* 02139, *USA*;
4. *Department of Physics, National University of Singapore, Singapore* 117542;
5. *Department of Pharmacy, National University of Singapore, Singapore* 117543)

Abstract: This article proposes a theoretical model of molecular sieving through repeated nanofilter arrays consisting of alternative deep and shallow regions. The role of configurational entropy, which arises from the inaccessibility of some configurations of the molecule in the confined space of nanochannel, is clarified explicitly. It is demonstrated that the configurational entropy difference of anisotropic biomolecules of different sizes dominates the complex partitioning of these molecules over the nanofilter array. In addition, the relationship between the effective mobility and the nanofilter geometries, molecular transport parameters, and the strength of electric fields are described rigorously. As an ex-

Received date: 2009-01-20; **Revised date**: 2009-04-30.

Foundation item: Supported by Singapore-MIT Alliance (SMA)-II, Computational Engineering (CE) programme.

ample, the mobilities for 50, 150 and 300 bp DNA molecules are calculated using this model, which matches the experimental data with a error less than 5%. This simplified model allows for fast analysis of nanofilter separation systems, without the need of complicated numerical simulations and physical experiments.

Key words: DNA; electrophoresis; nanofluidics; entropy barrier; biomolecule separation; ogston sieving

1 Introduction

The electrophoretic migration of polyelectrolyte in polymeric gels is a fundamental process in separation of biomolecules such as DNA, RNA and proteins. Gel electrophoresis has become a routine practice in biological related research and in pharmaceutical industries. These electric-field driven processes, as well as the transport of various charged macromolecules in biological systems, are controlled by the energy barriers induced by steric restrictions on the conformations of the macromolecules as they are navigating through pores[1-3]. Despite of its wide applications, the exact mechanisms involved in gel electrophoresis are far from satisfactory. One main obstacle is the randomness of the gel pores which is very difficult to characterize. As a potential substitutes of polymeric gel which contains random pores, patterned periodic regular sieving structures are found to be ideal for the study of molecular dynamics and electromigration of polyelectrolytes because the dimension of obstacles and channels can be precisely measured and controlled. Han and his group have used an array of microfabricated filtration device with regions of two different depths to study the motion of long DNA[4-5], rod-like short DNA[6-7] and small proteins[8]. For typical nanofilter array (used for separation of small biomolecules), the deep regions are in the scale of 1μm while the depth of the shallow regions is less than 100 nm. As the length of the DNA rods is in the same order or larger than the size of the nanofilter gap (depth of shallow region), the number of conformations that can be assumed in the nanofilter shallow region is reduced. This steric constraint decreases the orientational entropy and as a result increases the free energy of DNA. This entropy barrier is expected to play a dominant role in electrophoretic transport and separation of DNA molecules of different sizes.

Effective mobilities of various biomolecules and the size selectivity of such nanochannels have been studied largely based on mesoscopic models such as Brownian dynamics[9], and dissipative particle dynamics[10]. Unfortunately, these stochastic methods involve thermal fluctuations and require a long time to run. Many rounds of execution are usually required to get a useful average result. In addition, the input parameters of these methods are also difficult to determine for lack of well-established methods of coarse-graining. Therefore, these methods are generally used to elaborate relevant physical mechanisms. It is very difficult to make useful quantitative predictions using such simulation tools. In contrast to these stochastic simulations, we have conducted simulation study based on continuum transport model[11] and macrotransport theory[12]. Our method focuses on the behavior of group solutes and runs on a macroscopic scale. It was found, from the faithful reproduction the experimental results, that the electrophoretic separation of DNA molecules of different sizes is mainly accounted for by the entropy loss in the shallow regions.

In this paper, we will develop a theoretical model to obtain the explicit analytical expression of the mobility of anisotropic biomolecules in the nanofilters array. The effect of all the relevant

parameters such as the size and transport parameters of the molecule, the geometry of the nanofilter, and the strength of electric field, etc can be explicitly given. The experimentalists will have a handy formula to predict the mobility of the small rigid molecules without performing complicated numerical simulations.

2 Methods

2.1 Transport theory

Let $P(\boldsymbol{r},t)$ denote the probability density of a Brownian particle at point $\boldsymbol{r}=(x,y,z)$ of the channel at time t, the time evolution of $P(\boldsymbol{r},t)$ in the over-damped (strong friction) regime is governed by the Fokker-Planck equation[13],

$$\partial P(\boldsymbol{r},t)/\partial t=-\nabla\cdot\boldsymbol{J}(\boldsymbol{r},t)\ . \tag{1}$$

The probability flux $\boldsymbol{J}(\boldsymbol{r},t)$ is given by

$$\boldsymbol{J}(\boldsymbol{r},t)=-\frac{D_0}{k_{\mathrm{B}}T}[\nabla P(\boldsymbol{r},t)-P(\boldsymbol{r},t)\nabla U(\boldsymbol{r})]\ , \tag{2}$$

where k_{B} is the Boltzmann constant; t is the absolute temperature, and D_0 is the free-solution diffusion coefficient, $U(\boldsymbol{r})$ denotes the field of potential energy of the particle, as described in the next section.

2.2 Potential energy landscape

The potential energy of the nanofluidic system is composed of two terms, namely the electrostatic potential and the entropic potential. In a static electric field, the electrostatic potential of a charged particle in an electric field is $U_{\mathrm{e}}(\boldsymbol{r})=\tilde{q}\Phi(\boldsymbol{r})$, where $\Phi(\boldsymbol{r})$ is the electric potential and $\tilde{q}$ is the effective charge. The entropic potential energy of an anisotropic particle arises when some of its configurations are restricted in a solid wall. As defined here, local partition function $\kappa(\boldsymbol{r})$describes the probability of the molecule appearing at $\boldsymbol{r}$ compared with that in free solution. For a rigid molecule, the configuration is fully described by the molecule's angular orientations. In this case, $\kappa(\boldsymbol{r})$ is equal to the local orientational partition function $\rho_\theta(\boldsymbol{r})$, which is defined as the ratio of number of permissible orientations to the total number of possible orientations[14]. Then the configuration entropy is $U_s(\boldsymbol{r})=-k_{\mathrm{B}}T\ln\rho_\theta(\boldsymbol{r})$.

2.3 One-dimensional description

Since the transport of DNA molecules in the nanochannel as shown in Fig. 1 takes place mainly in the direction of the channel axis, the degree-of-freedom in the depth and width directions of the channel can be eliminated by proper projections.

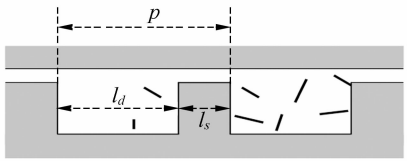

Fig. 1 Geometry of nanofilter array

As a result, the drift of biomolecules in the nanofilter array can be formulated as one-dimensional problem. Under such a formulation, the potential energy is formed by superposition of a sloped electrostatic potential and a stepped entropy barrier at the shallow region (see Fig. 2 and Fig. 3),

$$U(X)=\tilde{q}\Phi(X)-TS(X)\ . \tag{3}$$

In this one-dimensional formulation, the energy barrier ΔW is given by $\Delta W=-\ln\varepsilon K$. Here the energy barrier is caused by two factors: (a) the difference in the cross-sections of nanofluidic channel between the shallow region and deep region ($\varepsilon=d_s/d_d$); and (b) the partition coefficient (K) that accounts for the difference in the

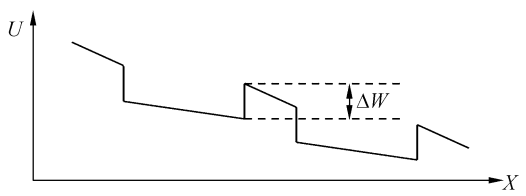

Fig. 2 Energy landscape of charged biomolecule in nanofilter array

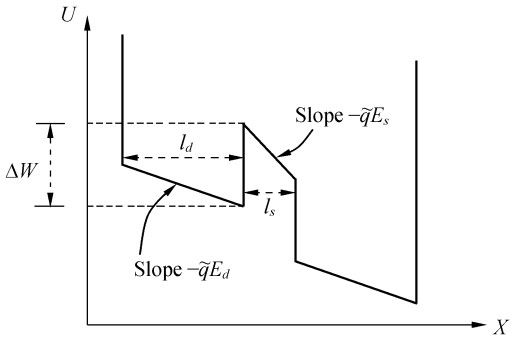

Fig. 3 Detailed energy profile over one unit of nanofilter

configurational freedom of the anisotropic particles in the shallow and deep regions of the nanofilter[14].

2.4 Mobility of a molecule in the nanofilter array

The phenomenological average velocity of the particle in the local intracellular potential field $U(x)$ as described by Eq. (3) can be obtained (by tedious derivation) by using a moment analysis method[15-16]

$$\mu=\frac{1}{1+\eta_t}\frac{(1+\nu)^2\varepsilon}{(\varepsilon+\nu)(1+\varepsilon\nu)}\mu_0, \tag{4}$$

with μ_0 being the free-solution electrophoretic mobility, and $\nu=l_s/l_d$ denoting the length ratio between the shallow and deep region of the nanofilter. The trapping effect due to the configurational entropy takes the form

$$\eta_t=\frac{(1-K)}{K}\frac{1-\varepsilon^2K}{1+\varepsilon\nu}\frac{(\varepsilon+\nu)}{\varepsilon}\times \frac{(1-e^{-\frac{\varepsilon}{\varepsilon+\nu}y_m})(1-e^{-\frac{\nu y_m}{\varepsilon+\nu}})}{y_m(1-e^{-y_m}!)}, \tag{5}$$

where $y_m=\tilde{q}E_{av}(l_s+l_d)/k_BT$ is the dimensionless potential drop over one unit of nanofilter.

As the main results of this paper, Eqs (4) and (5) provide key solutions to the molecular sieving in the nanofilter arrays as shown in Fig. 1. The role of configuration entropy is manifested in the partition coefficient K. If configurational term is negligible, the mobility expressed in Eq. (4) will reduce to a maximum sieving free ($\eta_t=0$) value. Trapping of molecule in the deep region would not take place, and no separation would be achievable if the molecules of interest possess similar value of μ_0, which is exactly the case for most DNA molecules.

3 Results and discussions

As an example, we analyzed the electrophoretic migration of short dsDNA molecules (of lengths 50 bp, 150 bp and 300 bp) in repeated nanofilter with geometry $l_s=l_d=0.5$ μm, $d_s=60$ nm and $d_d=240$ nm. Values of μ_0 are taken from experimental curves in Ref [17]. As a fitted value, the mobility of electroosmotic flow is $\mu_{eeo}=-2.81\times10^{-4}$ cm^{2}V^{-1} s^{-1} (more details on treatment of electroosmotic flow are described in Ref [10] and Ref [11]). The effective charge $\tilde{q}$ is calculated from μ_0 and D_0 as described in Ref [12]. Partition functions, which accounts for the effect of configurational entropy, are calculated numerically by enumeration of all the possible orientations of a rigid rod and check their permissibility, because these DNA molecules behave like short rods in aqueous solution at room temperature[11,14]. In this specific nanofilter, partition coefficient of a 300 bp DNA is −0.22, while the K values of 150 bp and 50 bp molecule are −0.52 and −0.89 respectively. In the limiting cases, an infinitely long molecule has $K=0$, while a point-size particle has $K=1$.

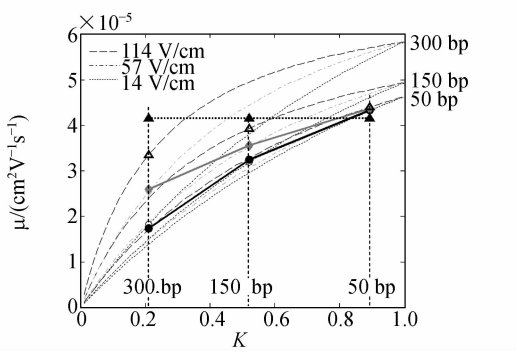

Fig. 4 Dependence of mobility μ of short DNA molecules on partition coefficient K under varied field strengths

In the next step, a series of curves of μ de-

pendent on K are plotted using Eq. (4) under a given voltage. For example, the three dashed blue curves in Fig. 4 represent the mobilities depending on K at voltage of 14 V/cm, based on the transport parameters of 300 bp, 150 bp and 50 bp respectively. There exist three different curves under a given electric field, because these short DNA molecules have different values of μ_0 and their maximum sieving mobilities ($K=1$, shown in the rightmost side of Fig. 4) are different (cf. Eq. (4)). The mobility of a particular molecule is obtained from its partition coefficient. If the mobilities of the molecules to be separated are well distributed, a good separation result is achievable. The plots shown in Fig. 4 also clarify the dependence of sieving effects on the electric fields. As we compare the curves of 15 V/cm and those under 57 V/cm and 114 V/cm, we found that the mobility increases almost linearly with K at low fields. In contrast, at high voltages, the mobilities of molecules increases to a maximum value at low range of K values and change very slowly in higher K regions, indicating a loss of the sieving effect. Among voltages investigated, 14 V/cm yields the best selectivity (shown as unfilled blue circles). At 114 V/cm, the separation is difficult as the mobilities of different molecules are close to each other. Theoretical results (hollow markers) agree well with experimental data (filled markers) under 14 V/cm and 57 V/cm fields. Under 114 V/cm, the molecules are not separated in the experiment. A single peak is obtained corresponding to a mobility shown in solid triangles.

4 Conclusions

We propose a theoretical model for the solutions the mobility in nanofilter arrays based on one-dimensional simplified model. In this model, the electrophoretic migration of the biomolecules is converted to the transport of point-sized particles through introduction of configurational entropy term. Then the three-dimensional problem is converted to one-dimensional macrotransport description by proper projection. The electric fields in the deep and shallow regions are assumed uniform respectively. In addition, a partition coefficient K (between shallow and deep regions) is introduced to account for the nonuniform distribution of configurational entropy. Based on such formulation, an analytical expression of the mobility is obtained with full consideration of field-driven drift and diffusion caused by the uneven concentration. The dependence of mobility on the geometry of channel, partition coefficient and electric field are all explicitly described. In a specific nanofilter array, different molecules take different values of configurational entropy, which manifest as different value of partition coefficients. The difference in diffusion coefficients produces different effective mobilities for these molecules and separation is achieved. In contract, without the configurational entropy, all the particles will run at their maximum sieving free mobility and the sieving effect does not exist. Practically, the explicit formulas derived here may serve as a handy tool for experimentalists in the analysis of the experiment data and in the optimization of the nanoarray structures and the field strengths without the need for complicated numerical simulations.

References:

[1] MUTHUKUMAR M. Mechanism of DNA transport through pores[J]. *Annual Review of Biophysics and Biomolecular Structure*, 2007, 36: 435-450.

[2] MUTHUKUMAR M, BAUMGÄRTNER A. Effects of entropic barriers on polymer dynamics

[J]. *Macromolecules*, 1989,22:1937-1941.

[3] SLATER G, GUILLOUZIC S, GAUTHIER M G, *et al*.. Theory of DNA electrophoresis (1999-2002 1/2) [J]. *Electrophoresis*, 2002,23:3791-3816.

[4] HAN J, TURNER S, CRAIGHEAD H. Entropic trapping and escape of long DNA molecules at submicron size constriction [J]. *Physical Review Letters*, 1999,83:1688-1691.

[5] HAN J, CRAIGHEAD H. Separation of long DNA molecules in a microfabricated entropic trap array [J]. *Science*, 2000,288:1026-1029.

[6] FU J, YOO J, HAN J. Molecular sieving in periodic free-energy landscapes created by patterned nanofilter arrays[J]. *Physical Review Letters*, 2006, 97:018103.

[7] FU J, SCHOCH R, STEVENS A, *et al*.. A patterned anisotropic nanofluidic sieving structure for continuous-flow separation of DNA and proteins [J]. *Nature nanotechnology*, 2007(2):121-128.

[8] FU J, MAO P, HAN J. A nanofilter array chip for fast gel-free biomolecule separation[J]. *Appllied Physics Letters*, 2005,87:263902.

[9] LAACHI N, DECLET C, MATSON C, *et al*.. Non-equilibrium transport of rigid macromolecules in periodically constricted geometries[J]. *Physical Review Letters*, 2007,98:098106.

[10] DUONG-HONG D, WANG J S, LIU G R, *et al*.. Dissipative particle dynamics simulations of electroosmotic flow in nano-fluidic devices [J]. *Microfluidics and Nanofluidics*, 2008(4):219-225.

[11] LI Z R, LIU G R, CHEN Y Z, *et al*.. Continuum transport model of Ogston sieving in patterned nanofilter arrays for separation of rod-like biomolecules[J]. *Electrophoresis*, 2008,29:329-339.

[12] LI Z R, LIU G R, HAN J, *et al*.. Transport of biomolecules in asymmetric nanofilter arrays[J]. *Analytical and Bioanalytical Chemistry*, 2009, 34:427-435.

[13] AJDARI A, PROST J. Free-flow electrophoresis with trapping by a transverse inhomogeneous field [C]. *Proceedings of the National Academy of Sciences, USA*, 1991,88:4468-4471.

[14] GIDDINGS J C, KUCERA E, RUSSELL CP, *et al*.. Statistical theory for the equilibrium distribution of rigid molecules in inert porous networks Exclusion chromatography[J]. *Journal Physical Chemistry*, 1968,72:4397-4408.

[15] BRENNER H, EDWARDS D. *Macrotransport Process* [M]. Boston: Butterworth-Heinemann, 1993.

[16] YARIV E, DORFMAN K D. Electrophoretic transport through channels of periodically-varying cross section[J]. *Physics of Fluids*, 2007,19: 037101.

[17] STELLWAGEN N, GELFI C, RIGHETTI P. The free solution mobility of DNA[J]. *Biopolymers*, 1997,42:687-703.

Authors' biographies:

LI Zi Rui, PhD, research fellow of the Singapore-MIT Alliance, Singapore, his research focuses include micro/nano-fluidics, biomolecule separation, theory of electrophoresis etc. **E-mail**: smalzr@nus.edu.sg

LIU G. R., Professor, Director of the Centre for Advanced Computations in Engineering Science, Department of Mechanical Engineering, National University of Singapore, Singapore. **E-mail**: mpeliugr@nus.edu.sg

HAN Joonyoon, Professor of the Massachusetts Institute of Technology, USA. **E-mail**: jyhan@mit.edu

WANG Jian-Sheng, Professor of the Department of Physics, National University of Singapore, Singapore. **E-mail**: phywjs@nus.edu.sg

Chen Yu Zong, Professor of the Department of Pharmacy, National University of Singapore, Singapore. **E-mail**:. phacyz@nus.edu.sg