Symmetry-Broken Reactant Motion upon Phase-Related Symmetrically Modulated Excitations: Application to Highly Selective Molecular Sorting

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This paper introduces a separation protocol relying on affinity chromatography that exhibits unprecedented selectivities. We submit the mixture contained in the separative medium to the simultaneous action of two symmetrically modulated excitations. The first is a uniform periodic field (e.g., electric field) with zero mean value, whereas the second is the periodic modulation of a thermodynamic parameter such as the temperature. Under appropriate tuning of the modulations with the dynamics of the discriminating chemical reaction, we predict a symmetry breaking of molecular motion: the mixture components that are addressed by their rate constants exhibit an oriented motion for a particular phase relation between the modulations of the field and the thermodynamic parameter. The resulting velocity of the mixture components depends on the rate constants and on a conjugated thermodynamic value such as the standard enthalpy of the discrimination process in the case of a temperature modulation. In particular, it may be possible to separate mixture components with identical rate constants. We use the present approach to design a protocol to sort nucleic acids by their sequence.

1. Introduction

Mixtures often result from syntheses, or extractions of natural products: most chemists face separations. To devise ingenious tools as well as to find optimal conditions to achieve separation lie at the heart of the analytical and preparative activity.[1] Essential is here to maximize selectivity[2] by identifying an appropriate discrimination process (for instance: a chemical reaction, the formation of a complex, the adsorption on a surface, ...). When dealing with mixtures of similar components such as members of a series, it may become difficult to find a process to selectively sort only one component. Then one is left to reveal at the most the differences existing among the mixture components with regards to the retained discrimination process.

Most separations are performed under quasistatic conditions: at any time, the system obeys the conditions of local and partial equilibrium with regards to the discriminative process.[3] Selectivity relies here on thermodynamics. For instance, the velocity of the mixture components in a chromatography column generally depends on the association constant for the stationary phase. As an alternative, we recently proposed to explicitly use the kinetics of the discrimination process in already well-tried separation techniques.[4−8] Emphasis on kinetic properties was obtained by periodically driving the separation medium away from equilibrium.[9−11] Application of a uniform time-periodic field with null average value maximizes the effective diffusion coefficient of a field-sensitive reactant when specific relations between its rate constants and the field properties are fulfilled.[4−6] Selective increase of the effective diffusion coefficient much beyond the intrinsic contribution[12] led us to introduce a novel chromatography protocol relying on enhanced diffusion.[4] In particular, we achieved to selectively sort from a mixture a dye that was addressed by its rate constants for association with α-cyclodextrin.[8] In principle, the expected advantage of basing a separation on kinetics is similar to working in a regime of kinetic control instead of a regime of thermodynamic control in preparative chemistry:[13,14] time becomes a relevant parameter to improve selectivity.

The present paper introduces a strategy that still further improves separation selectivity. We superimpose to the preceding field oscillation the modulation of a thermodynamically relevant parameter such as the temperature, the pressure or a reactant activity. Under appropriate tuning of the rate constants with the field frequency, we now get a symmetry breaking: field-sensitive reactants exhibit an oriented motion for a given phase relation between the modulations of the field and of the thermodynamic parameter. The corresponding behavior is reminiscent to the topic of Brownian motors.[15] In the context of separations, the behavior of a mixture component is governed by two different relaxations to the exerted perturbations and the larger selectivity here originates from the combination of two independent component-specific contributions to the overall response.

The paper is organized as follows. In the next section, we present the reaction−diffusion model. We first explain how we derive an analytic expression of the velocity of species with given kinetic properties in the presence of electric field and temperature oscillations. The choice of maximization conditions of this velocity is then discussed. Finally, the results are applied to sort nucleic acids by their sequence. The last section contains conclusion.

2. The Model

We consider a one-dimensional (1D) reaction−diffusion system submitted to a uniform, time-periodic electric field. The

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field-sensitive species C is supposed to react with a target P, producing Q, according to the reaction

$$P + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Q \tag{1}$$

that is common in Chemistry and Biology (formation of host−guest or ligand−receptor complexes, pairing between single stranded-DNA, ...). The rate constants k_1 and k_2 are respectively associated with the forward and backward reaction. The equilibrium constant of reaction 1 is given by $K = k_1/k_2$. In the following, we assume that the solution of C, P, and Q is ideal.

Adopting a macroscopic description, the concentrations of species C and Q submitted to reaction 1, respectively denoted by $C(x, t)$ and $Q(x, t)$, obey the following partial differential equations

$$\frac{\partial C(x,t)}{\partial t} = -k_1 P C(x,t) + k_2 Q(x,t) + D_C \frac{\partial^2 C(x,t)}{\partial x^2} - \mu_C E(t) \frac{\partial C(x,t)}{\partial x} \tag{2}$$

$$\frac{\partial Q(x,t)}{\partial t} = k_1 P C(x,t) - k_2 Q(x,t) + D_Q \frac{\partial^2 Q(x,t)}{\partial x^2} - \mu_Q E(t) \frac{\partial Q(x,t)}{\partial x} \tag{3}$$

where t is time and x is the spatial coordinate of the 1D-medium considered. In the right-hand side of eq 2, the two first terms originate from the chemical reaction 1. The third term is associated with diffusion, D_C and D_Q are the diffusion coefficient of species C and Q, respectively. The fourth term is related to the oscillating electric field where μ_C and μ_Q are the electrophoretic mobilities of species C and Q. We chose

$$E(t) = a \cos(\omega t + \phi) \tag{4}$$

with pulsation ω and phase ϕ. In the following, we admit that species C and Q experience differently the action of the electric field, either because they bear a different charge or because their size is different. We consequently consider that the mobility difference $\Delta\mu = \mu_C - \mu_Q$ does not vanish. We also suppose that species P has a constant uniform concentration thanks to appropriate exchanges with the exterior[16,17] or simply because P is in great excess. Then eqs 2 and 3 become linear and we introduce $\kappa_1 = k_1 P$ as an effective rate constant for the forward reaction 1.

In addition to the field oscillations, we impose a periodic modulation of small amplitude of temperature T. We choose a sinusoidal excitation with pulsation ω' and phase ψ and write

$$T = T_0[1 + \beta \cos(\omega' t + \psi)] \quad \text{with } \beta \ll 1 \tag{5}$$

Adopting the Eyring model[18] for the rate constants, $k_i = k_B T/h \exp(-\Delta_i G^{\dagger 0}/RT)$, we obtain

$$k_i = \frac{k_B T}{h} \exp\left(\frac{\Delta_i S^{\dagger 0}}{R}\right) \exp\left(-\frac{\Delta_i H^{\dagger 0}}{RT}\right) \tag{6}$$

where k_B is the Boltzmann constant, R is the individual gas constant, h is the Planck constant, $\Delta_i G^{\dagger 0}$ is the standard Gibbs free energy of activation at T^0 with $i = 1$ for the forward reaction and $i = 2$ for the backward reaction. $\Delta_i S^{\dagger 0}$, $\Delta_i H^{\dagger 0}$ are the corresponding quantities for entropy and enthalpy. Taking $\Delta_i S^{\dagger 0}$ and $\Delta_i H^{\dagger 0}$ constant in the relevant range, we expand the expression of the rate constant at first order in the perturbation. It reads

$$k_i = k_i^0 \left[1 + \left(\frac{\Delta_i H^{\dagger 0}}{RT_0} + 1\right)\beta \cos(\omega' t + \psi)\right] \tag{7}$$

where $k_i^0 = r_i \exp(-\epsilon_i)$ with $r_i = k_B T_0/h \exp(\Delta_i S^{\dagger 0}/R)$ and $\epsilon_i = \Delta_i H^{\dagger 0}/RT_0$.

3. Oriented Motion

Initially, a given amount N of the mixture of species C and Q at chemical equilibrium and at temperature T_0 is introduced at a given point of the medium, chosen as the origin. The initial condition reads

$$C(x, t=0) = \frac{1}{1 + \mathscr{K}^0} N\delta(x) \tag{8}$$

$$Q(x, t=0) = \frac{\mathscr{K}^0}{1 + \mathscr{K}^0} N\delta(x) \tag{9}$$

where $\delta(x)$ is the Dirac distribution and $\mathscr{K}^0 = K^0 P$ with $K^0 = k_1^0/k_2^0$. The 1D-medium is supposed to be infinite and the boundary conditions obey $\partial C/\partial x = \partial Q/\partial x = 0$ for $x \to \pm\infty$.

We define $I(t)$ as the total amount of species C in the medium at time t

$$I(t) = \int_{-\infty}^{+\infty} C(x,t)\, dx \tag{10}$$

$$N - I(t) = \int_{-\infty}^{+\infty} Q(x,t)\, dx \tag{11}$$

Conservation of matter has been used to deduce the total amount of species Q from $I(t)$. Integrating eq 2 over x from $-\infty$ to $+\infty$ and solving the differential equation obtained for $I(t)$, we find, at first order in β

$$I(t) = \frac{N}{1 + \mathscr{K}^0} + \frac{N\delta i_{\max}}{\sqrt{1 + (\omega'\tau_\chi)^2}} \left[\sin(\psi + \psi') \exp\left(-\frac{t}{\tau_\chi}\right) - \sin(\omega' t + \psi + \psi')\right] \tag{12}$$

with

$$\delta i_{max} = \frac{\mathscr{K}^0 \beta \Delta\epsilon}{(1 + \mathscr{K}^0)^2} \tag{13}$$

where $\Delta\epsilon = \epsilon_1 - \epsilon_2$ is the dimensionless enthalpy $\Delta_r H^0/RT_0$ of reaction 1, and $\tau_\chi = 1/(\kappa_1^0 + k_2^0)$ is the relaxation time of reaction 1. The angle ψ' obeys

$$\sin(\psi') = \frac{1}{\sqrt{1 + (\omega'\tau_\chi)^2}} \tag{14}$$

$$\cos(\psi') = \frac{\omega'\tau_\chi}{\sqrt{1 + (\omega'\tau_\chi)^2}} \tag{15}$$

The integral $I(t)$ is identical to the total amount in C that is obtained in a homogeneous mixture of C, Q, and P during relaxation experiments, that are used to measure the rate constants of reaction 1;[19] neither inhomogeneity of solute

distribution, nor field modulation alter the consequences of the periodic temperature modulation. The first term in the right-hand side of eq 12 is the equilibrium value of the C amount at T_0. The second term is the product of an amplitude $\delta I = N\delta i_{max}/[1 + (\omega'\tau_\chi)^2]^{1/2}$ by a function that evaluates the time-dependence of the system response to the temperature modulation: after a transient regime limited by the relaxation time of reaction 1, one enters into the forced regime with a sinusoidal response. At low enough field frequency ($\omega'\tau_\chi \ll 1$), many exchanges between the reactants and the products take place in average before any significant change of the temperature occurs: the system composition has enough time to relax to its instantaneous equilibrium value. The amplitude of the response is maximal and equal to $\delta I_{max} = N\delta i_{max}$ that can be easily derived from the van't Hoff equation ($\delta \ln \mathscr{K}^0 = \beta\Delta\epsilon$) with $I = N/1 + \mathscr{K}^0$. The temperature perturbation and the system response are in phase. An attenuation and a phase delay become significant in the system response around $\omega'\tau_\chi \approx 1$. Eventually, no chemical exchange takes place in average at high enough field frequency ($\omega'\tau_\chi \gg 1$): the system does not respond anymore to the temperature modulation.

We are here mainly interested in the mean position of the total amount of species C and Q. In other words, our aim is to determine the mean value of the position x, considered as a random variable distributed according to $(C(x, t) + Q(x, t))$

$$\langle x\rangle = \frac{1}{N}\int_{-\infty}^{+\infty} x(C(x, t) + Q(x, t))\, dx \tag{16}$$

Multiplying eqs 2 and 3 by x and summing the two equations, we deduce the following differential equation for the mean position $\langle x\rangle$

$$\frac{\partial\langle x\rangle}{\partial t} = -E(t)\left(\Delta\mu\frac{I(t)}{N} + \mu_Q\right) \tag{17}$$

Using the expressions of the field $E(t)$ and the integral $I(t)$ respectively given in eqs 4 and 12, the mean position $\langle x\rangle$ can be written as the sum of three components

$$\langle x\rangle = -\frac{a(\mu_C + \mathscr{K}^0\mu_Q)}{(1 + \mathscr{K}^0)\omega}[\sin(\omega t + \phi) - \sin(\phi)] - \frac{a\Delta\mu\delta i_{max}}{\sqrt{1 + (\omega'\tau_\chi)^2}}\left[\int_0^t \cos(\omega t + \phi)\sin(\psi + \psi')\exp\left(-\frac{t}{\tau_\chi}\right)dt - \int_0^t \cos(\omega t + \phi)\sin(\omega' t + \psi + \psi')\,dt\right] \tag{18}$$

The first line of the right-hand side of eq 18 is an oscillating term at pulsation ω. It is associated to the motion of the equilibrium populations in C and Q. Their respective relative proportions, $p_C{}^0 = 1/1 + \mathscr{K}^0$ and $p_Q{}^0 = \mathrm{K}^0/1 + \mathscr{K}^0$, move in phase with the electric field at the respective velocities $a\mu_C$ and $a\mu_Q$. The integral in the second line leads to transient terms that are negligible beyond the relaxation time of reaction 1. If the pulsations ω and ω' associated with the field and temperature oscillations are different, the integral in the third line leads to oscillating terms at pulsations $(\omega + \omega')$ and $(\omega' - \omega)$. These terms that oscillate at frequencies that differ from the excitation frequencies ω and ω' could be used to measure the rate constants of the reaction 1 by sensitive methods relying on synchronous detection. In the present context, the most interesting behavior is observed for $\omega = \omega'$. Beyond τ_χ, the mean position $\langle x\rangle$ obeys

$$\langle x\rangle = \frac{1}{2}a\Delta\mu\frac{\delta i_{max}}{\sqrt{1 + (\omega\tau_\chi)^2}}\sin(\psi + \psi' - \phi) \times t \tag{19}$$

when we omit the oscillating terms, i.e., for t multiple of the period $T = 2\pi/\omega$. Under these conditions, the mean position of the total amount of species C and Q, that is, the average position of the concentration profile $(C(x, t) + Q(x, t))$, moves at a constant velocity v that we write

$$v = \frac{\langle x\rangle}{t} = \frac{a\Delta\mu\beta\Delta\epsilon\mathscr{K}^0}{2(1 + \mathscr{K}^0)^2}\left\{\frac{\omega\tau_\chi\sin(\psi - \phi) + \cos(\psi - \phi)}{[1 + (\omega\tau_\chi)^2]}\right\} \tag{20}$$

when use is made of eq 13 for δi_{max} and eq 14 for ψ'.

Expression (19) of the position is appropriate to get some physical insight into the phenomenon. The second term of the right-hand side, $\delta i_{max}/[1 + (\omega\tau_\chi)^2]^{1/2} = \delta I/N$, is equal to the normalized amplitude of the modulation of the population of C, that is induced by the modulation of the temperature at pulsation ω (vide supra). It differs from zero only if $\Delta\epsilon \neq 0$. The distance covered by the amount δI during half a period is not necessarily retraced back during the following half period if $\Delta\mu \neq 0$. The resulting velocity correspondingly depends on $\delta I/N$, on the difference between the individual velocities of the C and Q states, $a\Delta\mu$, but also on the phase relation $(\psi + \psi' - \phi)$ between the system response to the temperature modulation, and the field periodic excitation. One has to notice that the physical origin of the present phenomenon differs from the cause of the dispersion that originates from application of a modulated field only.[4,6,8] In particular, velocity is acquired at any value of the field amplitude a, whereas a regime of strong field was required to observe the dispersion.

The superposition of an oscillating field and a temperature modulation at the same pulsation leads to an oriented motion for the reactant (C, Q) provided that (i) the two exchanging states C and Q experience differently the field, i.e., for $\Delta\mu \neq 0$, and (ii) the reaction 1 is not athermal, i.e., $\Delta\epsilon \neq 0$.[20] The corresponding symmetry breaking is remarkable if one considers that all of the excitations exerted on the system are uniform and symmetrical. Indeed in the presence of a modulated field, we only observed an isotropic effect: an increase in the apparent diffusion coefficient.[4,6,8] Oriented motion here originates from the double periodic excitations with a constant phase relation.

4. Optimization of Velocity in a Purpose of Separation

We first discuss the optimization conditions of the velocity of a given chemical species in view of its separation from a mixture. In standard separation techniques, a mixture component C is discriminated by a few independent quantities. For instance, only one is relevant in standard electrophoresis: its mobility μ. If C presents some affinity for the separative medium leading to the formation of a bound state Q, a thermodynamic quantity such as an association constant, K^0, should be additionally taken into consideration. In our previous work,[4,6,8] we introduced a protocol that improves the separation selectivity by making kinetics significant to control molecular sorting. Effective diffusion of the components (C, Q) has been shown to depend on three quantities: $\Delta\mu$, $k_1{}^0$, and $k_2{}^0$, instead of two. The present result brings some further improvement since the expression of the velocity given in eq 20 now depends on four quantities: $\Delta\mu$, $\Delta\epsilon$, $k_1{}^0$, and $k_2{}^0$. Thus, a separation relying on a difference

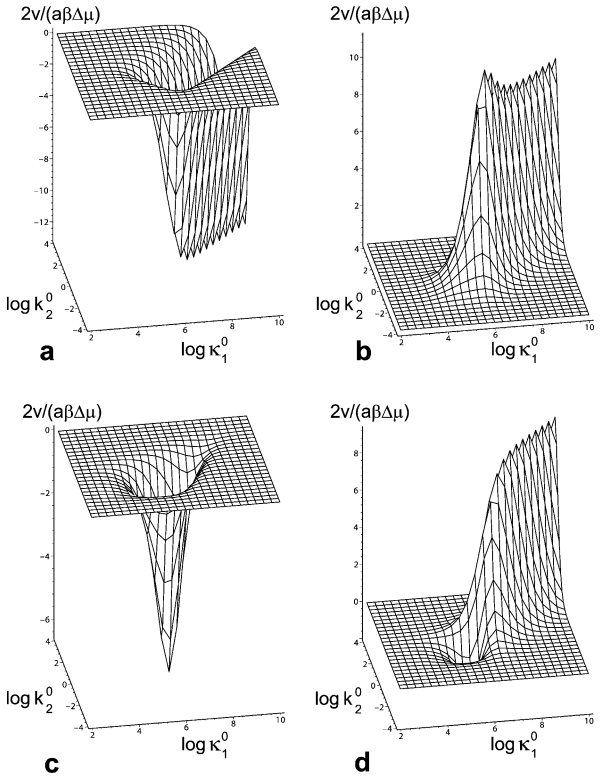

Figure 1. Scaled velocity $2v/a\beta\Delta\mu$ of the mean position of the total amount of species C and Q as a function of the rate constants κ_1^0 and k_2^0 in decimal logarithmic units for different phase differences between temperature and phase oscillations: (a) $\psi - \phi = 0$, (b) $\psi - \phi = \pi/4$, (c) $\psi - \phi = \pi/2$, (d) $\psi - \phi = 3\pi/4$. The preexponential factors r_1 and r_2 are variable, the other parameters are fixed at $\epsilon_1 = 8$, $\epsilon_2 = 60$, $\omega = 2\ s^{-1}$ and $P = 10^{-6}$ M.

of velocity should be highly selective. Difficulty arises from the dependence of the variables. Whereas the mathematical expression of v depends on $\Delta\epsilon$, k_1^0, and k_2^0, the physically relevant quantities are r_1, r_2, ϵ_1, and ϵ_2. The values of the control parameters, P, ω, and $(\psi - \phi)$, that optimize separation, are therefore not obviously chosen.

We consider a mixture of many similar couples (C, Q) differing only by the values of the set (r_1, r_2, ϵ_1, ϵ_2) that characterize the kinetics of exchange between state C and state Q at temperature T_0 in the presence of species P in excess. In particular, $\Delta\mu$ is assumed to be the same for all the couples. If this is not the case, one can always perform a first standard electrophoresis to separate the mixture components into families characterized by given values of $\Delta\mu$.

We first evaluate the significance of the phase difference $(\psi - \phi)$ in relation with the selectivity issue. Figure 1 shows representative cuts of the hypersurface $v(r_1, r_2, \epsilon_1, \epsilon_2)$ at fixed activation energies (ϵ_1, ϵ_2), i.e., at fixed $\Delta\epsilon$. Under these conditions, switching from the preexponential factors (r_1, r_2) to (κ_1^0, k_2^0) consists of a simple change of variables. As shown in Figure 1c, the range of (κ_1^0, k_2^0) values where v differs from zero is at the smallest when $(\psi - \phi)$ is close to $\pi/2$ modulo π. In contrast, nonvanishing values of the velocity are obtained over a larger (κ_1^0, k_2^0) range for other values of $(\psi - \phi)$ according to Figure 1a,b,d. For a separation purpose, the achievement of a narrow peak is crucial since it determines the selectivity of the procedure, i.e., the ability to separate couples (C, Q) with close properties. In the following, we therefore always choose the phase difference

$$\psi - \phi = \pi/2 \tag{21}$$

so that the expression of velocity reduces to

$$v = \frac{a\Delta\mu\beta\Delta\epsilon\kappa_1^0 k_2^0 \omega}{2(\kappa_1^0 + k_2^0)[(\kappa_1^0 + k_2^0)^2 + \omega^2]} \tag{22}$$

Note that eq 22 is symmetric by exchange of κ_1^0 and k_2^0 if $\Delta\epsilon$ is fixed.

We now consider the question of the extraction of a reference couple (C^R, Q^R) with predefinite values of the set (r_1^R, r_2^R, ϵ_1^R, ϵ_2^R) and consequently with predefinite rate constants $k_1^{0,R}$, $k_2^{0,R}$ and energy difference $\Delta\epsilon^R$.

We first address this issue by looking for appropriate values for P and ω that singularize at the most (C^R, Q^R) within the (C, Q) mixture components: we search P^R and ω^R such that the (C^R, Q^R) velocity is at the largest. The four independent physical quantities: r_1, r_2, ϵ_1, and ϵ_2 do not appear as independent variables in the mathematical expression of v given in eq 22. When looking for the extrema of $v(r_1, r_2, \epsilon_1, \epsilon_2)$, we find two sets of conditions

$$\partial v/\partial r_1 = \partial v/\partial r_2 = 0 \Leftrightarrow \kappa_1^0 = k_2^0 = \omega/2 \tag{23}$$

$$\partial v/\partial \epsilon_1 = \partial v/\partial \epsilon_2 = 0 \Leftrightarrow \kappa_1^0 + k_2^0 = \Delta\epsilon(k_2^0 - \kappa_1^0) = \omega \tag{24}$$

According to eq 23, a local maximum is reached in cuts of the phase space at fixed (ϵ_1, ϵ_2) for

$$P^R = \frac{1}{K^{0,R}},\ \omega^R = 2\kappa_1^{0,R} = 2k_2^{0,R} \tag{25}$$

Following eq 24 and provided that $|\Delta\epsilon^R| > 1$, a local maximum in cuts at fixed (r_1, r_2) is found for

$$P^R = \frac{1}{K^{0,R}}\frac{\Delta\epsilon^R - 1}{\Delta\epsilon^R + 1},\ \omega^R = 2\kappa_1^{0,R}\frac{\Delta\epsilon^R}{\Delta\epsilon^R - 1} = 2k_2^{0,R}\frac{\Delta\epsilon^R}{\Delta\epsilon^R + 1} \tag{26}$$

The condition $|\Delta\epsilon^R| > 1$ here makes precise how the thermodynamic constant K of the reaction has to be sensitive to temperature to maximize the (C^R, Q^R) velocity: the difference of dimensionless activation energy between the forward and backward reactions has to be larger than 1.

The two sets of conditions given in eqs 25 and 26 are compatible only if $|\Delta\epsilon^R| \gg 1$.[21] In this case, they are degenerate and reduce to eq 25 that only depends on ($k_1^{0,R}$, $k_2^{0,R}$) and not on all the (r_1^R, r_2^R, ϵ_1^R, ϵ_2^R) parameters. Thus, the choice of (P^R, ω^R) values maximize the velocity of a family of (C, Q) species characterized by ($k_1^{0,R}$, $k_2^{0,R}$). Nevertheless the maximized velocities associated with each member of this (C, Q) family depend on $\Delta\epsilon$

$$v_{\max} = \frac{a\Delta\mu\beta\Delta\epsilon}{16} \tag{27}$$

Since v_{max} explicitly depends on $\Delta\epsilon$, it is possible to separate (C, Q) species sharing identical rate constants ($\kappa_1^{0,R}$, $k_2^{0,R}$) obeying eq 25 but with a different value of $\Delta\epsilon$. Table 1 displays the dependence of $v_{\max}$ that is observed for mixture components of a ($\kappa_1^{0,R}$, $k_2^{0,R}$) family that differ in their activation enthalpy and activation entropy by ln10 at most in RT units. The different parameter values were taken in relation with the DNA example that is illustrated in the following.[22] The nine examined members have in common the same association constant $K^{0,R}$, as well as the same ($k_1^{0,R}$, $k_2^{0,R}$) set. Consequently, no separation method

TABLE 1: Scaled Velocity $-2v/a\Delta\mu\beta$ of DNA Strands Obeying Reaction 1 with Identical Rate Constants $k_1^{0,R}$ and $k_2^{0,R}$, but with the Terms r_i and $\exp(-\epsilon_i)$ Differing by 1 Order of Magnitude at Most from Those of the Reference[a]

$r_1, \epsilon_1, r_2, \epsilon_2$	$\Delta\epsilon$	$-2v/a\Delta\mu\beta$
$r_1^R, \epsilon_1^R, r_2^R, \epsilon_2^R$	$\Delta\epsilon^R$	6.50
$r_1^R/10, \epsilon_1^R - \ln(10), r_2^R/10, \epsilon_2^R - \ln(10)$	$\Delta\epsilon^R$	6.50
$10r_1^R, \epsilon_1^R + \ln(10), 10r_2^R, \epsilon_2^R + \ln(10)$	$\Delta\epsilon^R$	6.50
$r_1^R, \epsilon_1^R, 10r_2^R, \epsilon_2^R + \ln(10)$	$\Delta\epsilon^R - \ln(10)$	6.79
$r_1^R/10, \epsilon_1^R - \ln(10), r_2^R, \epsilon_2^R$	$\Delta\epsilon^R - \ln(10)$	6.79
$10r_1^R, \epsilon_1^R + \ln(10), r_2^R, \epsilon_2^R$	$\Delta\epsilon^R + \ln(10)$	6.21
$r_1^R, \epsilon_1^R, r_2^R/10, \epsilon_2^R - \ln(10)$	$\Delta\epsilon^R + \ln(10)$	6.21
$10r_1^R, \epsilon_1^R + \ln(10), r_2^R/10, \epsilon_2^R - \ln(10)$	$\Delta\epsilon^R + 2\ln(10)$	5.92
$r_1^R/10, \epsilon_1^R - \ln(10), 10r_2^R, \epsilon_2^R + \ln(10)$	$\Delta\epsilon^R - 2\ln(10)$	7.08

[a] Concentration of the target and pulsation of field and temperature oscillations are fixed at $P^R = 10^{-6}$ M and $\omega^R = 2s^{-1}$ for a reference couple characterized by $k_1^{0,R} = 10^6$ M^{-1} s^{-1}, $k_2^{0,R} = 1$ s^{-1}, $\epsilon_1^R = 8$, $\epsilon_2^R = 60$.

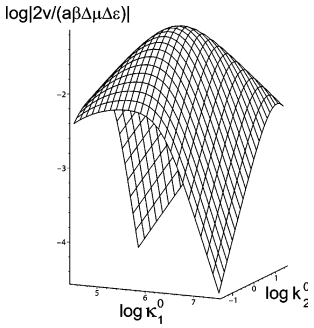

Figure 2. Decimal logarithm of scaled velocity $|2v/a\beta\Delta\mu\Delta\epsilon|$ versus $\log(\kappa_1{}^0)$ and $\log(k_2{}^0)$ at fixed $\Delta\epsilon$ for $\psi - \phi = \pi/2$, $\omega = 2$ s^{-1}, and $P = 10^{-6}$ M.

TABLE 2: Normalized Velocity $v\Delta\epsilon^R/v^R\Delta\epsilon$ of DNA Strands Obeying Reaction 1 with Rate Constants Differing from the Resonant Values by 1 Order of Magnitude at Most[a]

$k_1{}^0, k_2{}^0$	$v\Delta\epsilon^R/v^R\Delta\epsilon$
$k_1^{0,R}, k_2^{0,R}$	1.00
$k_1^{0,R}, 10k_2^{0,R}$ or $10k_1^{0,R}, k_2^{0,R}$	0.12
$k_1^{0,R}, 0.1k_2^{0,R}$ or $0.1k_1^{0,R}, k_2^{0,R}$	0.28
$10k_1^{0,R}, 10k_2^{0,R}$ or $0.1k_1^{0,R}, 0.1k_2^{0,R}$	0.20
$k_1^{0,R}, 10k_2^{0,R}$ or $10k_1^{0,R}, k_2^{0,R}$	0.12
$0.1k_1^{0,R}, 10k_2^{0,R}$ or $10k_1^{0,R}, 0.1k_2^{0,R}$	0.01

[a] Concentration of the target and pulsation of field and temperature oscillations are fixed at $P = 10^{-6}$ M and $\omega = 2$ s^{-1} for a reference couple characterized by $k_1^{0,R} = 10^6$ M^{-1} s^{-1}, $k_2^{0,R} = 1$ s^{-1}, $\epsilon_1^R = 8$, $\epsilon_2^R = 60$.

such as a classical affinity chromatography relying on K^0, nor the diffusive separation in the presence of a modulated field that depends on $(k_1{}^0, k_2{}^0)$[4,6,8] should achieve the separation of the mixture components. In contrast, the present approach discriminates among the nine considered components five subfamilies associated to the five possible $\Delta\epsilon$ values. Noticeably the reference component characterized by $(r_1^R, r_2^R, \epsilon_1^R, \epsilon_2^R)$ exhibits an intermediate velocity together with two other components. Three components move respectively more slowly and more quickly than (C^R, Q^R). Their velocities differ from $v_{\max}^R$ by 4.5% and 8.9% respectively.

In principle, components with rate constants that do not obey eq 25 may interfere with the preceding (C, Q) family: a large value of $\Delta\epsilon$ could compensate the decrease of v due to nonoptimized values of $(\kappa_1{}^0, k_2{}^0)$. As seen in Figure 2 and Table 2, couples (C, Q) with one rate constant equal to $k_1^{0,R}$ or $k_2^{0,R}$ will be the most limiting species. The main result is that the compensation would have to be envisaged only if some mixture components exhibit $\Delta\epsilon$ much larger than $\Delta\epsilon^R$. More precisely, a couple with $k_1{}^0 = k_1^{0,R}$ and $k_2 = 0.1k_2^{0,R}$ has a velocity 3.6 times smaller than $v_{\max}^R$ if $\Delta\epsilon = \Delta\epsilon^R$. Similarly, a component with $k_1{}^0 = k_1^{0,R}$, $k_2 = 0.1k_2^{0,R}$ and $\Delta\epsilon = 3.6\Delta\epsilon^R$ travels with the same velocity as the reference component.

The present protocol exhibits a high selectivity to perform separations: four independent parameters $\Delta\mu$, $\Delta\epsilon$, $k_1{}^0$, and $k_2{}^0$ characterize the motion of every reactant. In a mixture, one correspondingly anticipates an unprecedented dispersion of the velocities among the components. At the same time, the latter dispersion is not accompanied by a facilitated recovery of a desired species: it is difficult to singularize a given (C^R, Q^R) component by an extremal behavior. The excitation by an electric field at constant temperature led to maximize the apparent diffusion coefficient of a given (C^R, Q^R) couple, with predefinite rate constants $(\kappa_1^{0,R}, k_2^{0,R})$, by tuning P and ω. In the case of the superposition of field and temperature oscillations, a given (C^R, Q^R) couple exhibits a maximized velocity with respect to variables $\kappa_1{}^0$ and $k_2{}^0$, that still increases with $\Delta\epsilon$. In addition, couples with rate constants different from $(\kappa_1^{0,R}, k_2^{0,R})$ but with a large $\Delta\epsilon$ may travel at the same velocity as the desired couple. In a context of molecular sorting from unknown mixtures, successive separations relying on increasingly selective protocols should be favored: after a first separation to select mixture components with identical $\Delta\mu$, the separation protocol relying on field modulation only could be applied to sort with the $(\kappa_1^{0,R}, k_2^{0,R})$ criterium. The present protocol could be ultimately used to further refine the dispersion based on $\Delta\epsilon$, $k_1{}^0$ and $k_2{}^0$.

5. Application to the Detection of Single Nucleotide Polymorphism

The preceding considerations are not restrictive when one is interested in sorting components that exhibit the largest values of $\Delta\epsilon$. This makes the present protocol especially suited to analyze and to sort deoxyribonucleic acids. We now discuss the application of the separation method to the detection of single nucleotide polymorphism. In reference to the model presented previously, species C should be the variable single-stranded DNA to be probed, P an oligonucleotide probe with a complementary sequence to the portion of C to be analyzed, and Q the resulting double-stranded DNA. For short P oligonucleotide probes (typically 10 bases long), the forward rate constant $k_1{}^0$ is poorly sensitive to wrongly paired bases: it is expected to vary by less than a factor 10 upon variation in the C sequence.[23-25] In contrast, a single mismatch of bases already leads to a variation of the backward rate constant $k_2{}^0$ that can reach up to 3 orders of magnitude.[23-25] In relation to the activation energy, the hybridation is easy whereas the separation of two strands requires crossing over a high energy barrier: the activation energy difference $\Delta\epsilon$ between the forward and the backward reaction 1 is typically much larger than one. In addition, $\Delta\epsilon$ is at the largest for the perfect match pair denoted (C^R, Q^R). Under these conditions, the local maximization conditions given in eqs 23 and 24 are identical. In the following, the pulsation ω^R of the oscillations and the concentration of P^R are chosen such that the rate constants $k_1^{0,R}$ and $k_2^{0,R}$ of the perfectly matching strand C^R obey eq 25.

To check the sensitivity of the method, we consider the reference strand C^R and other single strands C with rate constants $k_1{}^0$ and $k_2{}^0$ that differ from $k_1^{0,R}$ and $k_2^{0,R}$ by only 1 order of magnitude. In a context of micromutation detection, such

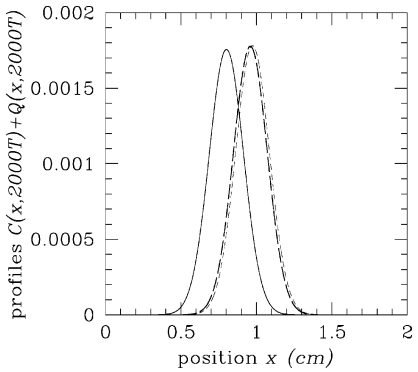

Figure 3. Total concentration profiles of species C and Q after 2000 periods of field and temperature oscillations. The results are given by the numerical integration of eqs 2 and 3 for $\Delta t = 0.01$ s, $\Delta x = 0.000005$ m and the following parameter values: $\beta = 0.01$, $D_C = D_Q = 10^{-10}$ m^2 s^{-1}, $a\mu_C = 1.7 \times 10^{-5}$ m s^{-1}, $a\mu_Q = 0.7 \times 10^{-5}$ m s^{-1}, $\omega = 2$ s^{-1}, $P = 10^{-6}$ M; the reference couple (C^R, Q^R) (solid line) obeys $k_1^{0,R} = 10^6$ M^{-1} s^{-1}, $k_2^{0,R} = 1$ s^{-1}, $\epsilon_1{}^R = 8$, $\epsilon_2{}^R = 60$; the two other couples considered are defined by $k_1{}^\circ = 10k_1^{0,R}$, $k_2{}^0 = 10k_2^{0,R}$ (long-dashed line); $k_1{}^\circ = k_1^{0,R}$, $k_2{}^0 = 10k_2^{0,R}$ (short-dashed line). Initially, a same amount of the different couples (C, Q) at chemical equilibrium is located in the middle of the medium.

couples are representative of mismatched (C, Q) pairs. In a context of sorting C strands containing a complementary sequence to that of P, such couples are the most disturbing species. In addition to ($k_1^{0,R}$, $k_2^{0,R}$), we consider four couples characterized by $K^0 \leq K^{0,R}$: ($10k_1^{0,R}$, $10k_2^{0,R}$), ($k_1^{0,R}$, $10k_2^{0,R}$), ($k_1^{0,R}/10$, $k_2^{0,R}$), and ($k_1^{0,R}/10$, $k_2^{0,R}/10$). In principle, one additionally expects $\Delta\epsilon < \Delta\epsilon^R$ in the series of these four couples. We used typical orders of magnitude for pairing between 9 bases-long single-stranded oligonucleotides[22] to calculate the values of $v\Delta\epsilon^R/v^R\Delta\epsilon$ predicted by eq 22 for these 5 cases (Table 2). These analytical predictions were compared to the results of numerical solutions of the partial differential equations governing the evolution of the concentrations. After introduction of discrete space and time variables, respectively, $x/\Delta x$ and $t/\Delta t$, where Δx is the length of a spatial cell and Δt, the time step, a simple finite-difference method of Euler type was used to solve the equations.[4] Some concentration profiles obtained are given in Figure 3. Solving numerically eqs 2 and 3 with the expression (6) for rate constants, allows us to check the validity of the approximations, i.e., the first-order expansion with respect to the amplitude of temperature oscillations β and the elimination of the transient and constant terms in the expression of the velocity. It also gives access to the broadening of the concentration profiles due to diffusion and dispersion. Indeed, eq 25 are identical to the resonance conditions for maximizing the (C^R, Q^R) dispersion.[4]

The mean positions of representative peaks are given in Figure 4. For $\beta = 10^{-2}$ and only after one period of oscillations, the different peaks travel with a velocity which agrees with the analytical prediction of eq 22 to within a few percent. For the parameter values associated with typical 9-bases DNA strands at a temperature close to 25 °C,[22] the velocity of the reference couple (C^R, Q^R) is of the order of $v^R = 0.5$ μm s^{-1} and is at least 3.6 larger than any other considered couple. Taking into account that a decrease of $k_2{}^0$ by a factor of 10 is the lowest anticipated limit, the detection of any micromutation or single nucleotide polymorphism, as well as C^R sorting by the P sequence should be easily achieved.

Figure 5 gives the evolution of the position variance: the broadening of the peaks associated with the different couples (C, Q) obeys a diffusion law. For the parameter values of Figure

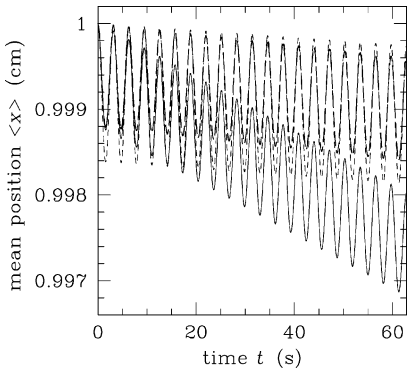

Figure 4. Time evolution of the mean position $\langle x \rangle$ of the reference couple (C^R, Q^R) and 2 other couples for the parameter values of Figure 3.

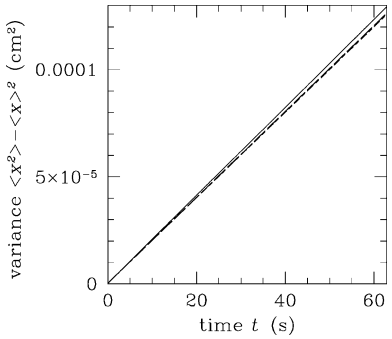

Figure 5. Same caption as Figure 4 for the variance of the position $\langle x^2 \rangle - \langle x \rangle^2$.

5, all the species have nearly the same diffusion coefficient close to $D_C = D_Q$: oriented motion has been achieved in the regime where apparent dispersion is governed by actual diffusion. As seen in Figure 3, the fastest couple (C^R, Q^R) keeps a peak as narrow as the other couples. We solved eqs 2 and 3 for different parameter values leading to apparent diffusion coefficients, that varies according to the values of the rate constants. Typical results are given in Figure 6. The values of the apparent diffusion coefficients, deduced from the slopes of the lines, agree to within a few percent with the theoretical predictions

$$D = D_C\frac{k_2{}^0}{\kappa_1{}^0 + k_2{}^0} + D_Q\frac{\kappa_1{}^0}{\kappa_1{}^0 + k_2{}^0} + \frac{(a\Delta\mu)^2\kappa_1{}^0k_2{}^0}{2(\kappa_1{}^0 + k_2{}^0)[(\kappa_1{}^0 + k_2{}^0)^2 + \omega^2]} \quad (28)$$

that we obtained in the absence of temperature oscillations.[4] Hence the expression of the apparent diffusion coefficient derived at a constant temperature T_0 can also be used in the case of a temperature modulation. It is therefore easy to derive the condition on field amplitude such that the broadening of the peaks is controlled by actual diffusion. For $D_C = D_Q$ this condition reduces to

$$a\Delta\mu \ll 4\sqrt{D_C\omega} \quad (29)$$

We checked that the small-field condition was obeyed for the parameters of Figure 5 with $\omega = 2$ s^{-1}, but not for those of Figure 6 with $\omega = 2 \times 10^{-3}$ s^{-1}.

In a purpose of separation or to facilitate a nonambiguous detection of an oriented motion, we introduce the time τ that is necessary for a peak to travel over a distance larger than its

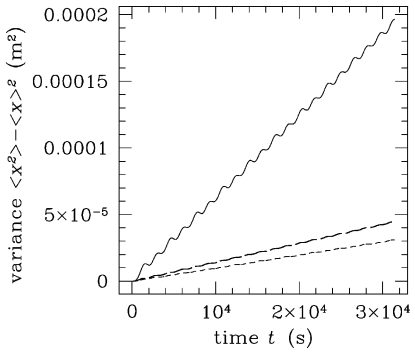

Figure 6. Time evolution of the variance of the position $\langle x^2\rangle - \langle x\rangle^2$ for the reference couple (C^R, Q^R) with $k_1^{0,R} = 10^5\ M^{-1}\ s^{-1}$, $k_2^{0,R} = 10^{-3}\ s^{-1}$, $\epsilon_1{}^R = 8$, $\epsilon_2{}^R = 60$ (solid line), and 2 other couples with $k_1{}^0 = 10\ k_1^{0,R}$, $k_2{}^0 = 10k_2^{0,R}$ (long-dashed line), $k_1{}^0 = k_1^{0,R}$, $k_2{}^0 = 10k_2^{0,R}$ (short-dashed line), for the following parameter values $\beta = 0.01$, $D_C = D_Q = 10^{-10}\ m^2\ s^{-1}$, $a\mu_C = 10^{-5}\ m\ s^{-1}$, $a\mu_Q = 0$, $\omega = 0.002\ s^{-1}$, $P = 10^{-8}$ M, $\Delta t = 0.1s$, $\Delta x = 10^{-5}$ m.

broadening. For the present method, τ obeys

$$\tau \simeq \frac{2D}{v^2} \tag{30}$$

where the diffusion coefficient and the velocity are respectively given by eqs 28 and 22. Two regimes are observed depending on the amplitude of the field oscillations. In a regime of weak field obeying eq 29, the broadening is controlled by intrinsic diffusion whereas, in a regime of strong field, the width of the peaks is controlled by the field amplitude. For the reference couple (C^R, Q^R) with rate constants that obey the conditions given in eq 23, the characteristic time reads

$$\tau_f \simeq \frac{2^9 D_C}{(a\Delta\mu)^2(\beta\Delta\epsilon)^2} \tag{31}$$

in a regime of weak field and for $D_C \simeq D_Q$. For the set of parameters displayed in Figures 3−5, $\tau_f \approx 2\ h$ is associated to a typical migration over 2 *cm*. In a regime of strong field, the characteristic time obeys

$$\tau_F \simeq \frac{2^4}{k_2(\beta\Delta\epsilon)^2} \tag{32}$$

In this regime, note that the choice of a large field amplitude increases the velocity v but does not decrease the characteristic time for separation. For the same set of parameters, the lower limit of τ_F is now 1 min that corresponds to a 30 μm oriented motion.

6. Conclusion

We introduced a chromatography protocol that exhibits an unprecedented selectivity. It is based on the oriented molecular motion that results from the application of two phase-related symmetrically modulated excitations: a uniform periodic field with average null value and a modulation of a thermodynamic parameter such as temperature. Whereas one or two parameters are generally involved to characterize every mixture component in current chromatography approaches, the present protocol relies on four independent parameters to determine the motion of the mixture components. Peak dispersion is at the largest. At the same time, the latter dispersion is not accompanied by a facilitated recovery of a desired species as we observed when the uniform periodic field was applied only. In the most general case, it is difficult to singularize a given component by an extremal behavior. This restriction disappeared when one is concerned with sorting mixture components with the largest response to the modulation in the thermodynamic parameter. Then it becomes even possible to separate mixture components that share the same rate constants for the discrimination process.

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(20) It is to be noted that, instead of temperature, we could modulate pressure or the concentration in species *P* and get an expression of velocity similar to eq 20. In fact, we consider temperature as the most efficient parameter to be modulated. In eq 20, the term $\Delta\epsilon$ responding to a modulation of amplitude β results from the dependence of the rate constants on temperature. In the case of pressure modulation, the dependence of the rate constants on pressure would involve the reaction volume instead of the reaction enthalpy in eq 20. The magnitude of the corresponding response term is usually smaller than $\Delta\epsilon$ and much lower velocities should be obtained for a modulation of same amplitude β. In the case of concentration modulation in an ideal solution, the velocity would depend on kinetic properties only through $\kappa_1{}^0$ and $k_2{}^0$ (in a nonideal solution, the modulation of *P* changes the rate constant values in a complex manner so as to forbid the derivation of an analytic expression of the velocity). In contrast to temperature modulation, selectivity with respect to $\Delta\epsilon$ would be lost.

(21) In the case of concentration modulation and if the solution is ideal, the velocity would depend on kinetic properties only through $\kappa_1{}^0$ and $k_2{}^0$ so that the velocity would admit a global extremum for the conditions given in eq 23. Optimization conditions would be more easily found.

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