Molecular sorting by stochastic resonance

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To sort a targeted species from a mixture, we introduce a procedure that relies on the enhancement of its effective diffusion coefficient. We use the formation of a host–guest complex between α -cyclodextrin and a dye to evidence the dye dispersion when the medium is submitted to an oscillating field. In particular, we demonstrate that the effective diffusion coefficient of the dye may be increased far beyond its intrinsic value by tuning the driving field frequency in the stochastic resonance regime. We use this effect to selectively sort from a mixture a dye that is addressed by its rate constants for association with α -cyclodextrin.

Sorting molecules from a mixture is an essential issue in chemistry (1). A radical answer consists in using “universal” chromatography columns, which would ideally resolve all of the mixture components. Then the counterpart is to reach a sufficient resolution. Affinity chromatography is another approach that can be envisaged when one is interested in extracting components that exhibit a given reactivity. For example, one can search in a mixture $\{C_i\}$ for a free molecule C_i that targets a receptor P to give a bound state C_iP according to the reaction

In the following, we consider association of dyes (C_i ; $i = 1$ or 2) with α -cyclodextrin (P) to form host–guest complexes (C_iP) (Fig. 1) (2–5). In a classic affinity chromatography, the average velocity $\bar{v}_i$ of a mixture component is ruled by the equilibrium association constant $K_i = k_i^f/k_i^b$, where the rate constants k_i^f and k_i^b are, respectively, associated to the forward and the backward reaction 1: Those components that do not react are easily discarded, whereas the others are separated according to their affinity for the target. With respect to “universal” columns, selective addressing with a chemical reaction reduces the mixture to a smaller pool, making separation less resolution-demanding.

To enlarge selectivity and facilitate molecular sorting that implies control of the motion of the mixture components, we propose a chromatography procedure that is related to affinity chromatography but that determines sorting by the kinetics of both binding and release, as described by k_i^f and k_i^b . In fact, the rate constants of the mixture components often differ to a larger extent than their association constants. For example, the dyes C_1 and C_2 exhibit a similar affinity for the α -cyclodextrin P : $K_1/K_2 = 0.34$ at 283 K (2, 3). In contrast, $k_1^f/k_2^f = 0.021$ and $k_1^b/k_2^b = 0.063$ at the same temperature. A chromatography based on differences in rate constants may therefore improve selectivity in comparison with traditional approaches that rely on differences in equilibrium properties (1, 6) (e.g., association constants). In addition, discrimination among components that exhibit the same association constant but different rate constants becomes possible.

To simultaneously emphasize on kinetic properties and induce molecular motion, we apply a uniform time-periodic field (e.g., an electric field) with zero average value having a period tuned to the dynamics of the reaction involved in selective addressing (here reaction 1) (7–9). We predicted that field-sensitive species

Fig. 1. Molecular formulas of the reactants submitted to reaction.

C_i submitted to reaction 1 with receptor P in excess do not move in average ($\bar{v}_i = 0$) but exhibit a diffusive behavior under the influence of the field (10, 11). The corresponding effective diffusion coefficient, $D_{\text{eff},i}$, is the sum of two contributions: $D_{\text{diff},i}$, which characterizes the stationary coupling between diffusion and reaction 1, and $D_{\text{disp},i}$, which is related to the field modulation. The diffusive term $D_{\text{diff},i} = p_{C_i}D_{C_i} + p_{C_iP}D_{C_iP}$ corresponds to a weighted average of the diffusion coefficients of C_i and C_iP , D_{C_i} and D_{C_iP} . The weights $p_{C_i} = 1/(K_i[P] + 1)$ and $p_{C_iP} = K_i[P]/(K_i[P] + 1)$, respectively, designate the stationary relative proportions of C_i and C_iP . They only depend on the thermodynamic constant K_i and the fixed receptor concentration $[P]$. The dispersive term $D_{\text{disp},i}$ results from coupling between the reaction 1 and the modulating field when the mobilities of C_i and C_iP , μ_{C_i} and μ_{C_iP} , are different (12–15). For a sinusoidal field modulation of amplitude a and frequency ω , $D_{\text{disp},i}$ can be written as

$$D_{\text{disp},i} = \frac{1}{2} \left[\frac{\kappa_i^f k_i^b a^2 (\mu_{C_i} - \mu_{C_iP})^2}{(\kappa_i^f + k_i^b)^3} \right] \left[\frac{1}{1 + \omega^2 / (\kappa_i^f + k_i^b)^2} \right], \quad [2]$$

with $\kappa_i^f = k_i^f[P]$. The first term in $D_{\text{disp},i}$ already exists by coupling reaction 1 with a constant field (12–16). It originates from the

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stochastic nature of the reaction **1**: All of the molecules of a given type do not experience identical individual trajectories, and they will be dispersed in the course of time around an average position $\bar{x}_i(t)$. The dispersion rate is maximized when $\kappa_i^f = k_i^b$ and at the largest for a slow reaction **1** (see supporting information, which is published on the PNAS web site). This result is expected from the law of large numbers: the ensemble behavior converges to the average behavior when the frequency of exchanges according to reaction **1** increases. The second term in $D_{\text{disp},i}$ is a cut-off function that cancels for a reaction that is too slow. It expresses that, for dispersion to occur, reaction **1** must take place during the delay when the concentration profiles of C_i and C_iP do not coincide: the relaxation time of reaction **1**, $\tau_{\text{rxn},i} = (\kappa_i^f + k_i^b)^{-1}$, must be shorter than half the period of the field $T = 2\pi/\omega$. For a sinusoidal periodic field, it is a classic Lorentzian.

In view of its square dependence on a , $D_{\text{disp},i}$ exceeds $D_{\text{diff},i}$ for large enough a values (10); in such a regime, dispersion is similar to forced diffusion. As shown in Fig. 2a, $D_{\text{disp},i}$ is a symmetric function of κ_i^f and k_i^b at a given period T . In view of the opposite dependence on κ_i^f and k_i^b of its two terms (see supporting information), $D_{\text{disp},i}$ exhibits one maximum whose coordinates are given by

$$\kappa_i^f = k_i^f[P] = k_i^b = N/T, \quad [3]$$

where N is a numerical factor depending on the nature of the periodic excitation (sinusoidal: $N = \pi$; square-wave: $N \approx 3.212$) (10). This maximum is interpreted as a stochastic resonance. Historically, stochastic resonance has been introduced in the case of a Brownian particle moving in a bistable potential in the presence of a periodic forcing (17, 18): The amplitude of the particle position, considered as a function of the diffusion coefficient, is found to be maximum when the characteristic time associated with the random process, the mean first passage time, identifies with the half-period of the forcing. The notion of stochastic resonance has been subsequently extended to other physical situations. It is used for phenomena involving random processes, when the output signal is optimized for a given value of the parameters associated with the stochastic processes. Here, the stochastic processes are the forward and backward reactions **1**, characterized by the two average life times of the free and bound states, $\tau_{C_i} = 1/\kappa_i^f$ and $\tau_{C_iP} = 1/k_i^b$. The external forcing is the periodic electric field. The apparent dispersion coefficient $D_{\text{disp},i}$, considered as a function of the rate constants κ_i^f and k_i^b , is maximum when the two time scales associated with the random processes coincide with the half-period of the field, as expressed by relations 3. Qualitatively, this stochastic resonance is understood as follows (10, 15). Thanks to reaction **1**, the molecules undergo random jumps between a more mobile state C_i and a less mobile state C_iP . When the jumps between the two states are in phase with the changes in direction of the field, the distance covered during a given half-period by the molecules in state C_i is not retraced when the field is reversed because they are in the less mobile state, C_iP . Hence, the couple (C_i, C_iP) whose rate constants obey relations 3 visits a more extended domain than the other couples: The apparent diffusion coefficient is optimized. The present result on a chemical system is reminiscent of diffusion enhancements of nonreactive particles that were obtained by matching the characteristic times associated to particle motion and to external forcing (19, 20).

Eqs. 2 and 3 are significant both for addressing and physically separating on kinetic criteria a given mixture component C_i^R . First, $D_{\text{disp},i}$ explicitly depends on the rate constants k_i^f and k_i^b and not only on K_i . In addition, $D_{\text{eff},i}^R$ can be always made the largest among the $D_{\text{eff},i}$ if C_i^R is "resonant," i.e., if the receptor concentration and the field period are chosen so that the rate constants associated with C_i^R obey the resonance conditions given in Eq. 3. A sorting protocol can now easily be designed: (i) spot the

Fig. 2. Normalized dispersive contribution to the effective diffusion coefficient of a species C_i submitted to reaction **1** in the presence of a square-wave periodic field. (a) Theoretical variation with $\kappa_i^f = k_i^f[P]$ and k_i^b (field period $T = 90$ s) (see supporting information). (b) Experimental variation with $[P]$ (filled circles, C_1 , 298 K; empty circles, C_2 , 283 K); also shown are the fits relying on the theoretical expression. $D_{\text{eff},i}$ is measured as described in Fig. 4. $D_{\text{diff},i}$ and $a(\mu_{C_i} - \mu_{C_iP})$ were evaluated from the literature (2, 3) and from our measurements. Solvent: acetic acid/boric acid/phosphoric acid buffer (pH 12, 0.15 M).

mixture $\{C_i\}$ in a medium with the appropriate receptor concentration $[P]_i^R = 1/K_i^R = k_i^{b,R}/k_i^{f,R}$; (ii) apply a uniform periodic field with $1/T_i^R = k_i^{b,R}/N$ and a such that $D_{\text{eff},i}^R \approx D_{\text{disp},i}^R$; and (iii) collect the targeted species C_i^R in the tails of the diffusion profile (Fig. 3a). We describe in Fig. 3b the related protocol that we used for our experiments. This sorting strategy should be very selective. Many C_i will not react with P ; for these, $D_{\text{eff},i} \approx D_{C_i} \ll D_{\text{eff},i}^R$. For the remaining putative guests of P , Fig. 2a shows that a difference in the rate constants κ_i^f and k_i^b by one order of magnitude decreases $D_{\text{disp},i}$ by a factor exceeding 3.5. In addition, C_i^R is here systematically located in the most favorable position of the "elution" profile: the leading edges; sorting is strongly facilitated. In current approaches of affinity chromatography, diffusion that is too large would hamper resolution between mixture components eluted at different velocities; in contrast, the absence of average displacement of the mixture here makes

Fig. 3. Possible chromatography procedures relying on the selective enhancement of the effective diffusion coefficient of a targeted species in a mixture. In the considered example, an equimolar mixture $\{C_1, C_2\}$ is either spotted in (a, the initial condition is a Dirac function) or put into contact with (b, the stationary boundary condition is a Heaviside function) the chromatography medium that is subsequently submitted to the application of the periodic field under resonant conditions for the targeted component (here C_1). After integer numbers of field cycles, the concentration profiles of C_1 and C_2 only reflect differences in effective diffusion. C_1 , which diffuses faster than C_2 , can easily be collected either at or beyond a given position. In our experiments, we measured the total amount of dyes that penetrated into the gel ($x \geq 0$) after one cycle of field in the geometry displayed in b. Solid line, $[C_1] + [C_1P]$; dotted line, $[C_2] + [C_2P]$; dashed line, purity $\Pi = ([C_1] + [C_1P])/([C_1] + [C_1P] + [C_2] + [C_2P])$. The figures were obtained after one cycle of field by using resonant conditions for C_1 at 283 K ($T_1^R = 3.212/k_1^b = 510$ s and $[P]_1^R = 1/K_1 = 3$ mM; applied voltage 150 V) (see supporting information). The zone indicated with the arrow marks the location where $\Pi = 90 \pm 1\%$.

the increase of the effective diffusion not a drawback, but a key to success.

Materials and Methods

Materials. The azo dyes C_1 (3'-ethyl-4'-hydroxyphenylazo-1-naphthalene-4-sulfonate) and C_2 (3',5'-diisopropyl-4'-hydroxyphenylazo-1-benzene-4-sulfonate) were synthesized according to classic procedures (21). α -cyclodextrin P was from Avocado (Heysham, U.K.), and low electroendosmosis agarose was from Roche Diagnostics. The Britton-Robinson (BR)

buffer (acetic acid/boric acid/phosphoric acid; pH 12 at 0.15 M) was prepared according to ref. 22.

Measuring the Velocities and the Effective Diffusion Coefficients of the Dyes. Measurements were performed in a small transparent electrophoretic cell (see supporting information). Two reservoirs containing platinum electrodes are linked by a 1×1 -mm² square glass capillary (VitroCom, Mountain Lakes, NJ) immersed in a thermostated glass cell. The capillary is filled with a hot ($>88^\circ\text{C}$) 1% (wt/wt) agarose solution prepared by dissolving the agarose powder in BR buffer containing P at the appropriate concentration. After gelification, the capillary is mounted in the electrophoretic cell, and the electrode reservoirs are filled with BR buffer. One reservoir of the electrophoretic cell is adjusted at $3 \cdot 10^{-4}$ M in dye. Then a constant electric field is applied with a dc voltage generator (E385, Consort, Turnhout, Belgium) to bring the concentration step of dye to the middle of the capillary. At that point, the electric field is either turned off or changed for the alternating regime according to the experiment. The frequency of the square-wave field is driven by the data acquisition program, which allows synchronous detection. The transmitted image from a thin channel parallel to the capillary containing the dye (x axis) is recorded as a function of time (y axis). The dye absorbs the light from a blue-light-emitting diode (silicon carbide blue light-emitting diode), and the remaining light is collected with a charge-coupled device camera (Model 4913, Cohu, San Diego) connected to a computer. The observed field is about 20 mm, with a spatial resolution equal to 34 ± 0.5 μm per pixel. Average values, as well as standard deviations, of the dye velocities can be extracted from observing the location of the front of the concentration step as a function of time.

Molecular Sorting. One reservoir of the electrophoretic cell is adjusted at $3 \cdot 10^{-4}$ M in C_1 and C_2 . Then one cycle of the periodic electric field (voltage 150 V) is applied under the resonant conditions for the considered dye ($T_1^R = 510$ s and $[P]_1^R = 3$ mM for C_1 , and $T_2^R = 31$ s and $[P]_2^R = 1$ mM for C_2): A uniform constant electric field determines displacements of the same amplitude from left to right for the first half-period $T_1^R/2$ and from right to left for the second half-period $T_1^R/2$. After one cycle, the reservoir initially containing the dyes is washed and filled with pure buffer. A constant electric field (150 V) is then applied to elute the dyes remained in the gel. The relative concentrations in C_1 and C_2 are directly determined from the gray levels in the recorded image of the elution profile because the molar absorption coefficients of both dyes are essentially identical in the range of excitation wavelengths of the light-emitting diode.

Results and Discussion

We evaluated the present sorting strategy by using the model chemical system displayed in Fig. 1. With identical charge but different size and shape, the dyes C_i and their complexes C_iP differ in their electrophoretic mobilities; a square-wave periodic electric field (a in the $3,000 \text{ V} \cdot \text{m}^{-1}$ range) was used to induce dispersion. In relation with Fig. 2a, we measured the effective diffusion coefficients of C_1 and C_2 at different concentrations in P to alter the value of $\kappa_i^f = k_i^f[P]$; in fact, k_i^b is fixed for a given dye, and the analysis of $D_{\text{disp},i}(\kappa_i^f, k_i^b)$ here reduces to the study of $D_{\text{disp},i}(\kappa_i^f)$. The k_i^b values available in the literature (2, 3) were used to determine the field period fixed during the whole series of experiments involving a given dye; we chose the specific value $T = 3.212/k_1^b$ given in Eq. 3 (90 s for C_1 at 298 K and 31 s for C_2 at 283 K).

To evidence at the same time the diffusive motion and to measure $D_{\text{eff},i}$, we used a Fourier analysis of the time evolution of step profiles of concentration of the dyes. These profiles were

Fig. 4. Extraction of the effective diffusion coefficients of C_i . (a) Typical record of the concentration profile of a dye (x axis) submitted to a square-wave periodic field as a function of time (y axis). The dye (dark zone) is first introduced from the left of the picture and moved up to $x = 10$ mm by application of a constant electric field. The field is then switched to a periodic square wave (Left). One observes a periodic motion in time of the front and a diffusion of the profile $C_i(x,t)$ (Right; Top Right after correction for electrophoretic migration). (b) The concentration profile of C_i obeys $\partial C_i(x,t)/\partial t = D_{\text{eff},i} \partial^2 C_i(x,t)/\partial x^2$. By using a discrete Fourier transform, the preceding equation transforms into $\partial \tilde{C}_i(q,t)/\partial t = -D_{\text{eff},i} q^2 \tilde{C}_i(q,t)$ with $q = 2\pi n/L$, where L designates the width of the acquisition window and n designates the index of the Fourier mode. Its solution is $\tilde{C}_i(q,t) = \tilde{C}_i(q,0) e^{-D_{\text{eff},i} q^2 t} = \tilde{C}_i(q,0) e^{-t/\tau_n}$ with $\tau_n = 1/[D_{\text{eff},i} (2\pi n/L)^2]$. The decays of the Fourier modes (Inset) are fit by an exponential law to extract the $1/\tau_n$ values. The quadratic dependence of $1/\tau_n$ on n provides the effective diffusion coefficient $D_{\text{eff},i}$.

observed with a camera in a thermostated transparent electrophoretic cell containing a 1% agarose gel to avoid electroosmosis. The spatial Fourier transform of the recorded pictures was analyzed after correction for electrophoretic migration (Fig. 4a). The evolution of the different Fourier modes was fit by exponential decays (Fig. 4b). The quadratic dependence of the corresponding relaxation times on the indices of the Fourier modes demonstrated that the motion of the dyes was diffusive and additionally provided $D_{\text{eff},i}$ (Fig. 4b).

Fig. 2b displays the experimental normalized dispersive contributions to the effective diffusion coefficients $(D_{\text{eff},i} - D_{\text{diff},i})/[a(\mu_{C_i} - \mu_{C,P})]^2$ on $[P]$. The fits relying on the theoretical predictions with only the rate constants k_1^f and k_1^b are satisfactory for both dyes. They give $k_1^f = 5.5 \pm 0.4 \text{ M}^{-1}\cdot\text{s}^{-1}$ and $k_1^b = 5.3 \pm 0.5 \cdot 10^{-2} \text{ s}^{-1}$ for C_1 at 298 K, and $k_2^f = 76 \pm 6 \text{ M}^{-1}\cdot\text{s}^{-1}$ and $k_2^b = 5.5 \pm 0.7 \cdot 10^{-2} \text{ s}^{-1}$ for C_2 at 283 K. The latter values compare well with the results reported in the literature at the same temperature under similar conditions of pH: $k_1^f = 6 \text{ M}^{-1}\cdot\text{s}^{-1}$ and $k_1^b = 3.5 \cdot 10^{-2} \text{ s}^{-1}$ for C_1 at 298 K, and $k_2^f = 100 \text{ M}^{-1}\cdot\text{s}^{-1}$ and $k_2^b = 0.1 \text{ s}^{-1}$ for C_2 at 283 K (2, 3). In particular, the position of the maximum of the dispersion coefficient $D_{\text{disp},i}(\kappa_i^f)$ reasonably obeys the resonance condition 3. We observe in Fig. 2b $[P]_1^{\text{D}_{\text{disp},\text{max}}} = 7.5 \text{ mM}$ and $[P]_2^{\text{D}_{\text{disp},\text{max}}} = 1.1 \text{ mM}$; $1/K_1 = 5.8 \text{ mM}$ and $1/K_2 = 1.0 \text{ mM}$ were, respectively, anticipated from the literature values. These results provide the first observation in chemistry of dispersion in an oscillating field (10, 12–15). Above all, they demonstrate by validating our theoretical calculations that one can considerably increase the effective diffusion coefficient of any reactant much beyond its intrinsic value $D_{\text{diff},i}$ (23) by tuning the frequency of the driving field in a regime of stochastic resonance.

As mentioned above, selective forcing of diffusion opens a road for sorting a molecule from a mixture $\{C_i\}$. We performed a series of experiments aimed at demonstrating that either C_1 or C_2 can be sorted at will from an equimolar mixture of C_1 and C_2 . We adopted the geometry displayed in Fig. 3b and measured the ratios of the integral amounts A_i in dyes trapped in the gel after one cycle under resonating conditions for both C_1 and C_2 . Increasing the number of cycles enlarges the sorting yield but does not significantly improve the purity $\Pi = ([C_1] + [C_1P])/([C_1] + [C_1P] + [C_2] + [C_2P])$; one cycle is both fast and efficient. Under switching the resonance conditions, we obviously achieved sorting of different targeted species: we found $A_1/A_2 = 2.0 \pm 0.2$ when C_1 was resonant, whereas $A_2/A_1 = 1.7 \pm 0.2$ when C_2 was resonant. These values satisfactorily compare with the theoretical predictions relying on literature data (2, 3) and on our experimental determinations of the diffusion coefficients and mobilities (see supporting information): $A_1/A_2 = 3.5$ and $A_2/A_1 = 1.8$. Note that the selectivity could be improved by increasing the field amplitude: $D_{\text{eff},i}^R/D_{\text{eff},i}$ is at the largest ($D_{\text{disp},i}^R/D_{\text{disp},i}$) for large a . With our set-up, we obtained $D_{\text{eff},1}^R/D_{\text{eff},2} = 9$ (C_1 resonating) and $D_{\text{eff},2}^R/D_{\text{eff},1} = 3$ (C_2 resonating); 11 and 13 could be reached by multiplying a by a factor 3. Nevertheless, it is clear that the enrichment in the resonant species is already significant for analysis by comparing the initial mixture and the enriched fraction. In fact, Fig. 3b suggests that much better results could be obtained by performing analyses at specified locations within the gel. For instance, Π as large as $90 \pm 1\%$ could be obtained at normalized concentrations equal to 0.30 at the position $x = 1.6 \text{ mm}$ (C_1 resonating) and 0.05 at $x = 0.45 \text{ mm}$ (C_2 resonating). Such numbers suggest a possible implementation on an integrated analytical lab-on-a-chip, using high-resolution mass spectrometry for detection (24).

The present approach for molecular sorting seems especially attractive when the receptor P is a biomolecule. Availability in low amount would not necessarily be limiting in view of the absence of average displacement of the mixture during sorting; a tiny amount of separative medium is sufficient. The docking process 1 could determine major changes of C_i mobilities leading to large values of $D_{\text{disp},i}$. Finally, fast sorting could be performed. In the situation illustrated in Fig. 3b, the whole sorting operation takes $T_1^R = 3.212/k_1^{b,R} \approx 500 \text{ s}$. T_1^R only slightly exceeds the chemical relaxation time $\tau_{\text{rxn},1}^R = 1/(\kappa_1^{f,R} + k_1^{b,R}) = 1/(2k_1^{b,R}) \approx 80 \text{ s}$ that is the minimal time for sorting the targeted species C_i^R with reaction 1 in classic affinity chromatography. In particular, T_i^R always remains lower than the delay

to reach the thermodynamic regime in the presence of unknown mixtures. In fact, sorting duration may become significant when taxonomy issues possibly leading to identifying dangerous species are involved. As an example, one could apply the present protocol to probe nucleic acids with short sequences of complementary oligonucleotides. Literature data (25–27) suggest that possible mismatches could be detected at

room temperature in a few seconds to be compared with the hours required with available approaches (28).

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