ENHANCED ELECTROPHORETIC TRANSPORT VIA NOISE-SYNCHRONIZED NANOSCALE ENTROPIC TRAPPING

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ABSTRACT

Macromolecules confined within nanoporous surroundings experience entropic trapping (ET) when their dimensions approach the average pore size, leading to emergence of transport behavior that can be immensely beneficial (e.g., a counterintuitive trend of increasing separation efficiency with DNA size during gel electrophoresis) [1]. But the noisy uncorrelated process by which the embedded macromolecules discretely hop from pore to pore contributes additional dispersion that detrimentally impacts most practical applications. Here show how the same dynamics governing phenomena as diverse as global climate change and sensory perception can be exploited to direct macromolecular transport through nanoporous surroundings. We demonstrate this in the context of gel electrophoresis by establishing a resonance condition that synchronizes the otherwise noisy uncorrelated motion of DNA between pores in the matrix. Surprising consequences include simultaneous transport of different-sized molecules in opposite directions, and a counterintuitive inverted size dependence of separation efficiency. We further show how DNA binding interactions can be sensitively probed by exploiting conformation dependence of resonance. These phenomena can be easily accessed in ordinary hydrogels (as opposed to idealized planar nanomachined topologies), offering a direct pathway to implement them in a host of useful settings.

KEYWORDS

Entropic trapping, electrophoresis, single molecule analysis.

INTRODUCTION

Macromolecules confined within nanoporous surroundings experience entropic trapping (ET) when their dimensions approach the average pore size [2], leading to emergence of transport behavior that can be immensely beneficial (e.g., a counterintuitive trend of increasing separation efficiency with DNA size during gel electrophoresis). Early gel electrophoresis studies helped identify qualitative features of DNA migration in the ET regime, but a straightforward physical description was slow to emerge and it was not immediately apparent how these effects could be harnessed to achieve improved separation performance. This outlook changed dramatically with the introduction of so-called "nanofilter" devices where the hydrogel matrix was replaced by arrays of nanomachined fluidic channels whose height h periodically alternates between deep wells ($h \sim R_g$) and narrow slits ($h < R_g$) (Fig. 1a). In addition to enabling DNA migration to be directly probed in the ET regime, the greatly simplified geometry consisting of only two length scales (i.e., the heights of the deep and narrow regions) made it possible to develop improved physical models describing the observed phenomena. But hydrogel matrices remain extremely attractive because they are easy to prepare and adapt for routine use, and because their nanoscale pore structure can be readily tailored by controlling polymerization conditions (Fig. 1b).

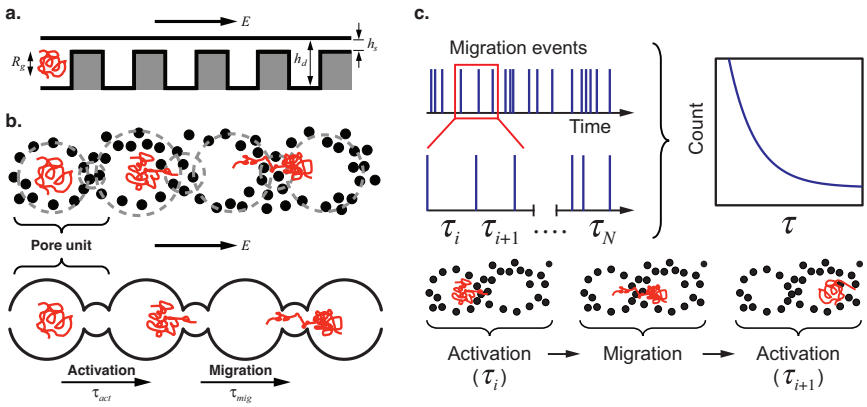

Figure 1. (a) "Nanofilters" create an idealized 2-D path consisting of alternating deep wells ($h_d \sim R_g$) and narrow slits ($h_s < R_g$). (b) We model hydrogels as a random ensemble of loosely and densely crosslinked regions (i.e., a pseudo-nanofilter; dots represent gel fibrils, not to scale). (c) Stochastic resonance can be applied by noting that the distribution of activation times between pore-to-pore hops (migration events) acts analogously to noise, suggesting synchronization is achievable by modulating the electric field at a period tuned to the activation timescale.

But the noisy uncorrelated process by which the embedded macromolecules discretely hop from pore to pore contributes additional dispersion that detrimentally impacts most practical applications. In recent studies, we have discovered that both the source and solution to previous difficulties encountered in exploiting ET effects for

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electrophoretic manipulation in gels are intimately linked to the inherent dynamics of the underlying activated transport mechanism. By modulating the applied electric field at a period tuned to the characteristic activation timescale (e.g., by periodically switching it on and off), a stochastic resonance condition is established that synergistically combines accelerated mobility and reduced diffusion (Fig. 1c).

EXPERIMENT

DNA gel electrophoresis experiments were performed using a previously described microchip-based platform [1]. Photocurable crosslinked polyacrylamide gels were prepared using Duracryl (30 %T, 2.6 %C; Proteomic Research Services) and the photoinitiator (Solution B) from a ReproGel DNA sequencing gel kit (GE Healthcare). For example, a 30 µL quantity of 6 %T gel contained 6 µL of the 30 %T gel stock solution, 4 µL of deionized water, 18.5 µL of Solution B, and 1.5 µL of stock 10x TBE buffer (Extended Range; Bio-Rad). An electrophoresis microchip (300 – 500 µm wide, 40 µm deep microchannel) was filled with the gel mixture, and the gel interface was defined by masking with opaque tape. Polymerization was performed under UV light (EXFO OmnniCure S1000) for 10-12 min. Separations were performed using a 100 bp double-stranded DNA ladder (Bio-Rad). Samples were prepared by mixing 5 µL of the DNA ladder, 8.5 µL of YOYO-1 intercalating dye diluted to one tenth of the stock concentration, and 1.5 µL of the anti-photobleaching agent β-mercaptoethanol. Time varying electric fields with a square wave profile were applied using a function generator (Agilent 33220A) interfaced with a voltage amplifier (Trek Model 603). Data analysis was performed using MATLAB. Data are averages over at least three experiments.

RESULTS AND DISCUSSION

In initial studies, we have succeeded in rationally identifying conditions favorable for ET-dominated transport in photopolymerized crosslinked polyacrylamide hydrogels by quantitatively characterizing their pore size distributions and directly probing the scalings of mobility and diffusion with DNA size. ET under a constant electric field yields an inverted trend of increasing separation resolution with DNA size (the opposite of what is conventionally seen), with a caveat that the absolute resolution values are below those obtainable under reptation-dominated transport (Fig. 2a, blue points; the resolution parameter R expresses the difference in distance traveled by two neighboring species relative to the sum of the half-widths of their associated peaks). But R increases significantly when the electric field is cyclically switched on and off at a period of $\Gamma = 5$ ms, reaching levels that match or exceed those obtained in the reptation regime while simultaneously preserving the anomalous increasing size dependence (Fig. 2a, red data). The enhancement coincides with a distinct mobility maximum at $\Gamma \sim 5$ ms that is most pronounced in the 500 to 1000 bp range, shifting toward lower Γ for smaller fragments (Fig. 2b). This effect is synergistically coupled with reduced diffusion relative the continuous field case (Fig. 2c).

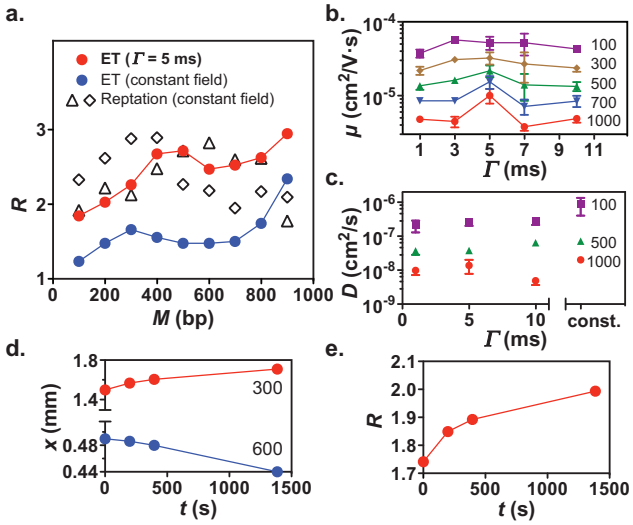

Figure 2. (a) Microchip gel electrophoresis of a 100 bp dsDNA ladder in the ET regime (imposed by UV curing the matrix at 625 mW cm^{-2}) shows enhanced separation resolution when the electric field is cyclically switched on and off at $\Gamma = 5$ ms (●, $E_{avg} = 10$ V cm^{-1}; it was necessary to apply an E_{avg} value less than 15 V/cm to remain within the ET regime) versus application of a continuous field (●, $E = 15$ V cm^{-1}). An inverted dependence of R on DNA size is also evident, as compared with the constant or decreasing size dependence of R in gels where transport is reptation-dominated (△, ◇; UV cured at 5 and 100 mW cm^{-2} respectively, $E = 15$ V cm^{-1}). (b) Measurements of electrophoretic mobility versus Γ display a distinct peak in the vicinity of 5 ms that is most pronounced at DNA fragment lengths 500 bp and above. (c) The periodically applied electric field also yields a reduction in experimentally measured diffusion coefficients relative to the constant field case. (d) Bi-directional transport of 300 and 600 bp DNA fragments is achieved by applying alternating forward and backward pulses of an oscillatory electric field with period and pulse times are selected to optimize resonance in each species. The net result is positive mobility of the leading species ($\mu_{300} = 1.5 \times 10^{-5}$ cm^2 V^{-1} s^{-1}) and negative mobility of the trailing species ($\mu_{600} = -0.5 \times 10^{-6}$ cm^2 V^{-1} s^{-1}), (e) yielding a continual increase in resolution with no increase in separation length.

Emergence of a size-dependent optimal modulation period also makes it possible to induce bi-directional transport of different sized DNA fragments. This is achieved by periodically alternating between an electric field in the forward direction with Γ tuned to match resonance of the faster (leading) species, and a field in the reverse direction at a resonant period associated with the slower (trailing) species (Fig. 2d). The combined effect drives forward transport of the leading species (positive mobility) while the trailing species travels backward (negative mobility). Bi-directional transport makes it possible to achieve a state in which separation resolution continually increases over time—an effect that is precisely tunable to isolate DNA in a specific size range within a very short separation distance (Fig. 2e). This mode of electric field actuation (i.e., cyclically switching the electric field on and off) is distinct from conventional pulsed field gel electrophoresis where the electric field direction is changed in a way that periodically induces re-orientation of very long DNA fragments. Our method is also distinct form zero-integrated field approaches where the electric field strength and duration change during the course of the separation and the period is not tuned to match a system timescale.

In addition to its utility for enhanced separations, stochastic resonance can be harnessed as a sensitive probe of DNA binding interactions. Complexation between DNA and binding agents (small molecules, proteins, adducts, etc.) is of considerable interest owing to their role in governing a myriad of biological functions including packaging, transcription, and regulation. These interactions are also important from a pharmaceutical standpoint as potential drug targets. Our method is enabled by exploiting the fact that the conditions under which resonance emerges strongly depend on the DNA coil size. Therefore, binding interactions become visible as a distinct peak in electrophoretic mobility occurring at a conformation-dependent electric field actuation period. We tested this approach by applying it to study binding interactions between a 600 bp dsDNA fragment with the intercalating chemotherapy drug compound daunomycin. After injection into the electrophoresis microdevice, a DC potential was first applied to drive the DNA into the gel and acquire the constant field mobility. The field was then cyclically switched on and off at periods ranging from 2 to 7 ms at 0.5 ms increments. The microchip was scanned along its length at 3 points in time under each field actuation condition, enabling the mobility to be extracted from the displacement as a function of time. A complete set of data is acquired in ~ 20 min.

Our results reveal that electrophoretic mobility of a 600 bp dsDNA fragment is significantly altered upon binding with daunomycin (Fig. 3). The effect is dramatic, with the peak mobility of the 600 bp complex shifting to be nearly equivalent to that of a native 300 bp fragment, laying a foundation for a "hyper mobility shift" assay as a diagnostic tool to reveal the presence of binding interactions and the extent of binding (i.e., from shifts in the resonant period and mobility value). These measurements also enable structural information (persistence and contour lengths) to be extracted by correlating the period at maximum mobility with conformational characteristics of the DNA complex. Remarkably, this information is obtainable by performing a single microchip electrophoresis experiment, providing potential for parallelized screening that is challenging to achieve by conventional methods. There is no inherent lower limit on the DNA size that can be interrogated, as opposed to single-molecule studies which require large DNA (λ-phage or longer).

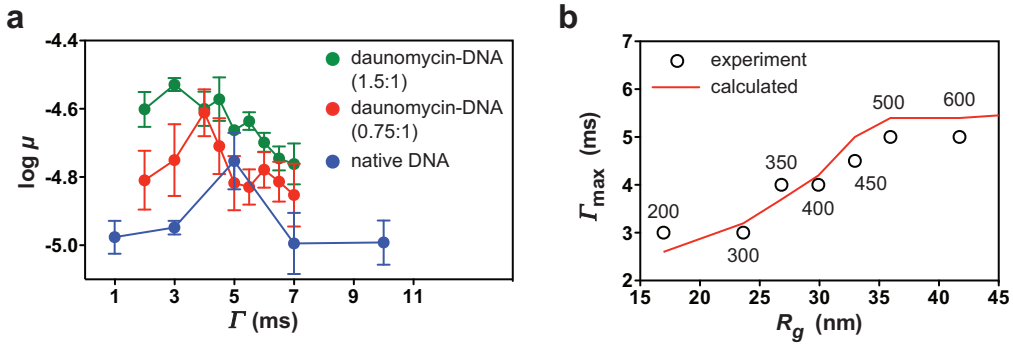

Figure 3. (a) Effect of DNA-daunomycin binding on resonant electrophoretic transport. The resonant mobility peak (Γ_{max}) is shifted to lower periods and higher values upon binding, reflecting the more compact size of the bound complex (data for two different daunomycin:bp loadings are shown). (b) Measuring Γ_{max} over a range of native DNA fragments with known length enables molecular size of the bound complex (R_g) to be determined. Experimental measurements (open symbols with DNA length in bp indicated) are in agreement with our transport model predictions (solid line). Once this relationship is established, contour and persistence lengths of the DNA complex can be extracted from the Γ_{max} data in (a).

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