Nanofluidic Entropophoresis

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Entropophoresis [1] is an emerging nanofluidic method using nanoscale confinement to control the spontaneous transport, separation, and concentration of DNA molecules and other nanoscale objects. Complex confinement schemes [2] and resulting free energy landscapes can be designed to entropically manipulate the otherwise random diffusion of nanoscale analytes in solution. Such nanofluidic structures and methods constitute a new approach to using engineered thermodynamic processes to automate the operation of lab-on-a-chip technology, for both practical analytical applications and related fundamental investigations.

In an initial demonstration of entropophoresis, DNA molecules descended a nanofluidic staircase, a slitlike nanofluidic channel with a depth profile approximated by a staircase function with 30 steps with widths of 4 μm and depths that ranged from (4 ± 4) nm to (342 ± 4) nm (average ± standard deviation) [1]. The staircase is the first nanofluidic structure with a complex three dimensional surface, fabricated from the top down by grayscale photolithography and a nanoscale pattern transfer process [2]. A high ionic strength electrophoresis buffer resulted in a short Debye length of <1 nm beyond which electrostatic interactions were screened, favoring the steric aspect of the complex confinement of DNA by the nanofluidic staircase. Linear DNA molecules of a standard length (48.5 kilo base pairs [kbp]) dyed with YOYO-1 were studied primarily, in addition to circular DNA molecules of comparable length (42.2 kbp), as well as longer concatemers and shorter fragments of linear DNA. The system was initialized by electrokinetically transporting DNA molecules into the shallow region (to the top) of the nanofluidic staircase, which resulted in a starting state of strong confinement and high confinement free energy. The externally applied electric field was then nulled, and the system evolved spontaneously towards thermodynamic equilibrium, as observed by widefield epifluorescence microscopy.

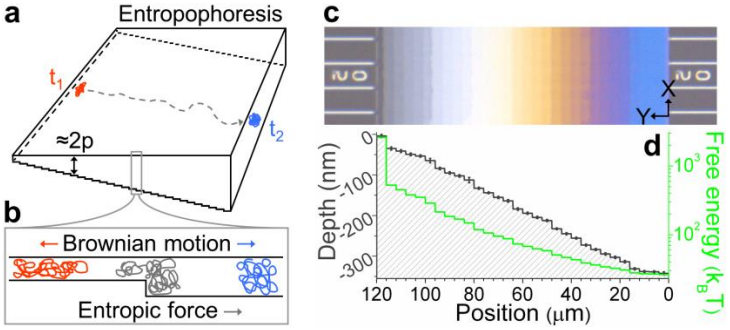

Figure 1. Nanofluidic entropophoresis [1]. a, To initialize the system, a DNA molecule was electrokinetically transported to the top of a nanofluidic staircase with step depths that spanned 2*p*, where *p* is the DNA persistence length of ≈51 nm. At t_1, the externally applied electric field was nulled, and the DNA molecule descended the staircase by entropophoresis. At t_2, the DNA molecule concentrated at the bottom of the staircase in an entropic trap. **b,** The DNA molecule diffused randomly across the width of each step of the nanofluidic staircase. Entropic forces ratcheted the DNA molecule at the edges between adjacent steps. **c,** Each step of the staircase is apparent as a distinct color due to white light interference in a brightfield optical micrograph of the empty device. **d,** The staircase (gray, average ± standard deviation) established a complex free energy landscape (green) for confined DNA molecules of standard length (48.5 kbp) dyed with YOYO-1.

DNA molecules diffused randomly on each step of the staircase, until encountering an edge between adjacent steps. Across the step edge, DNA molecules sampled a change in confinement free energy by diffusive fluctuations in conformation. This resulted in an applied entropic force that ratcheted the otherwise random diffusion of the DNA molecule away from the step edge and towards the deeper of the two steps. In this way, DNA molecules descended the nanofluidic staircase in a stepwise approach towards thermodynamic equilibrium, concentrating near the bottom of the staircase in an entropic trap.

DNA length and topology significantly influenced the rate of transport by entropophoresis, introducing the possibility of separating a mixture of DNA molecules by these characteristics. Larger DNA molecules, despite having a smaller diffusivity, descended the nanofluidic staircase faster than smaller DNA molecules, due in part to the decreased free diffusion distance between step edges. Larger DNA molecules that continually spanned more than one step width were subjected to the continual application of entropic forces, eliminating the relatively slow free diffusion component of entropophoresis and resulting in faster transport. Long DNA molecules that simultaneously spanned multiple step edges were subjected to the simultaneous application of multiple entropic forces, greatly increasing the rate of transport. Linear DNA molecules entropophoresed faster than circular DNA molecules of comparable length, due in part to the lack of a topological connectivity constraint and the resulting larger sizes of linear DNA for similar contour lengths, as measured by the component of the radius of gyration projected parallel to the slit surfaces. These different entropophoretic trajectories highlight the complex interactions of molecular size, topology, and diffusivity with the topography of nanoscale confinement. Related studies of the size variation [3, 4] and diffusion [5] of DNA in nanofluidic slits are essential to understand and optimize these interactions.

Heuristic measures of performance were developed for this initial demonstration of entropophoresis, involving linear DNA of a standard length in a high ionic strength electrophoresis buffer. DNA molecules were transported over the greatest distance ever induced by nanofluidic confinement at a rate that varied between one and two orders of magnitude faster than free diffusion. Structural control over DNA transport was most efficient at nanofluidic slit depths of $<3p$, where p is the native DNA persistence length. Less flexible nanoscale objects, such as nanoparticles, can also be manipulated by nanofluidic structures with complex three dimensional surfaces [2]. However, steric size exclusion plays a more significant role in the nanofluidic separation and characterization of such analytes [6].

Confinement and transport are fundamental to nanofluidics. Complex three dimensional nanofluidic structures [2] and entropophoresis [1] represent and integrate the state of the art in both. Beyond the analytical processes outlined here, DNA entropophoresis in complex nanofluidic confinement has great utility for basic studies in polymer physics [3, 4] and in fluctuations of nanoscale systems. Indeed, such studies are essential to provide a firm foundation upon which to develop thermodynamically automated lab on a chip technology for practical applications.

Disclaimer

Certain commercial equipment, instruments, or materials are identified to adequately specify the experimental procedure. Such identification implies neither recommendation nor endorsement by the National Institute of Standards and Technology nor that the materials or equipment identified are necessarily the best available for the purpose.

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