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Transport and Reactivity in Small Arrays of Nanopores

Introduction

Molecular transport in structures of nanometer (1 nm < *d* < 100 nm) characteristic dimensions is a critical component of a large number of separation technologies and sensor paradigms. Thus, it is critical to establish intelligent control of molecular transport in space and time at small length scales. Intelligent control implies materials and structures that can sense molecular characteristics, *e.g.* size, charge, molecular shape, *etc.*, and then generate control signals that control transport based on those molecular characteristics. Specifically we seek to manipulate, (separate, isolate, react, detect) low-mass samples with the same precision and level of control currently possible with bench-scale samples by combining microfluidic and nanofluidic structures to achieve *integrated microfluidics* capable of addressing the challenging problems posed by *multi-dimensional separations and analysis* with low mass samples. We are addressing this problem by pursuing two broad objectives: (1) development of an understanding of differential transport of ions based on charge and molecular shape through optical and electronic measurements of ion transport in single, high-aspect-ratio cylindrical nanopores; and (2) exploiting intrapore affinity-strength molecular recognition events to understand the manner in which ligand binding occurs in geometrically-confined spaces under flow.

Single Nanopore Transport

We are concentrating on the transport of polyelectrolytes and make extensive use of DNA as a model system, because (1) its properties are extremely well characterized, and (2) there is a facile fluorescence staining procedure which yields single molecules carrying multiple fluorescent labels, thereby simplifying the single pore optical transport studies. The common geometry for these experiments employs a nanocapillary membrane (*Ncap* = 1 (single pore) or ~103 in nanocapillary array membranes, NCAMs). Electrokinetic injection can be followed down to the single molecule level. In single pores we observe that: *a*) the apparent concentration of injected DNA does not appear to vary systematically with injection potential, consistent with saturating the forward bias potential for this single pore transport experiment; and *b*) the increases in fluorescence intensity observed for successive injections require increasingly longer times to reach steady state, consistent with formation of a DNA depletion region in the source channel.

Reactivity in Nanopores

This set of experiments asks if the confined spaces inside nanochannels can significantly impact reaction kinetics. Most enzymatic reactions in free solution are diffusion-limited; however, this may not be the case in a confined nanochannel environment. To address this problem NCAM-linked microfluidic structures were used to study the turnover of horseradish peroxidase (HRP) when the enzyme is bound to the interior surface of a cylindrical nanopore. Reaction kinetics were characterized semi-quantitatively by comparing measured product generation rates to predictions of finite element kinetic modeling. These rates are then compared to the same reactions on the surfaces of microfluidic channels and in free solution.

To implement these studies we prepare NCAMs with HRP immobilized inside the nanopores, but rigorously removed from the exterior surfaces. Then, the nanopores supporting fluidic communication between the source and receiving channels were subjected to either diffusive or electrokinetic forcing conditions. During the diffusive phases, fluorescence intensity increases, resulting from the build-up of product in the receiving channel. Then, during the electrokinetic injection phase it drops precipitously, back to near-background levels. As the injection-recovery cycles proceed, a repetitive low signal-high signal pattern appears.

In order to understand this behavior more clearly, finite element simulations - combining electrokinetic flow, diffusion, and enzymatic reaction occurring only at the surface of a nanopore - were performed. The simulations show a number of interesting features. First, independent of transport mechanism or reaction rate, product concentration reaches a maximum in the middle of the nanopore, and decreases gradually away from the nanopore. Second, comparing reactions with the same reaction rate, reactions under diffusion are always larger than that produced by electrokinetic injection. Additionally, as the reaction rate becomes larger, the resorufin concentration profiles under the two conditions become more similar. Finally, the simulation produces a concentration profile that can be compared with measured concentrations in the receiving channel. Comparing the measured fluorescence intensities in the quasi-steady state regions of the recovery phases in the data produces a resorufin concentration in the receiving channel of ~300 nM. Since transport in the recovery phase is purely diffusive, the observed concentration is properly compared with the diffusive simulations to bracket the overall Eley-Rideal rate constant at a value 103 M-1s-1 < *ktot* < 104 M-1s-1. These values may be compared to free solution values.

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