VOLTAGE GATED NANOFLUIDIC CHIP FOR PROTEIN CAPTURE, AMPLIFICATION, AND RELEASE

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ABSTRACT

We report on a nanofluidic ‘field-effect’ gated chip, with addressable gate electrodes to capture, amplify, and release a model protein, Bovine Serum Albumin (BSA). Fluidically isolated gates, embedded strategically along the length of the nanofluidic chip, when addressed facilitates generation of localized and spatially varying electric-field, driving protein amplification, *via* electro-migration, as opposed to diffusion driven amplification observed in existing state-of-art systems. A complementing numerical model was solved to capture the changes to the local space-charge density due to gating inside the nanofluidic chip, driving pre-concentration of BSA at various segments.

INTRODUCTION

Protein trafficking through nanometer scaled channels are ubiquitous in several biological processes. While microfluidic platforms have shown potential for protein pre-concentration *via* high-voltage concentration polarization methods [1], nanofluidic systems provide exclusive size and charge based selectivity to sense, trap, and amplify proteins for potential applications in proteomics, bioanalysis, clinical diagnosis, gene sequencing, and drug development. Nanofluidic platform can therefore serve as a central platform to trap proteins in their native environment. Voltage gated nanofluidic devices, geometrically analogous to solid state field effect transistors, are an attractive platform to re-configurably modulate the concentration of charged proteins *in vitro* [2].

Previously efforts to demonstrate field-effect control of proteins were achieved inside a nanofluidic channel consisting of an embedded gate electrode patterned over the entirety of the nanochannel [2, 3]. The net protein pre-concentration is therefore governed by the magnitude of electrostatic interaction between the charge protein and the entire charged nanofluidic wall. By modulating the polarity of the gate voltage, effective channel surface charge was altered, regulating protein transport inside the nanochannel in a diffusion limited pathway [2].

The purpose of this paper is to reduce the time-scale for protein pre-concentration by driving the amplification process *via* electro-migration as opposed to diffusion. To do so, individually addressable gate electrodes were patterned using lithography techniques on top of an SiO₂ dielectric, that fluidically isolated the metal gate from the nanochannel. The gate electrodes were located strategically along the length of the nanofluidic chip. When addressed with varying magnitudes of gate potential, localized and spatially varying electric fields (e-field) were generated inside the nanofluidic chip, driving protein amplification *via* electromigration.

THEORY

Ion concentration inside nanofluidic channels can be modified by altering the wall surface charge [2-9]. The potential drawback of modifying the surface chemistry of the wall *via* passive surface modification techniques is an irreversible, permanently altered wall surface charge state [4]. The wall surface charge can also be transiently controlled *via* application of transverse potential gradient through a metal gate which is typically separated from the nanofluidic channel walls *via* a thin strip (50–100 nm) of dielectric [5, 6]. With ψ and ϕ defined as potential distribution function inside the nanochannel and dielectric respectively, and ψ_0 defined as the

surface potential of the wall, the boundary condition at the surface of the dielectric-electrolyte interface in the presence of a gate (along y , Fig. 1) is given by Equations 1 - 3 [7],

$$-\epsilon_0 \epsilon_d \frac{d\phi}{dy} + \epsilon_0 \epsilon_f \frac{d\psi}{dy} = -\sigma_s \quad (1)$$

$$\phi = (\psi_0 - V_g) \frac{y}{\delta} + \psi_0 \quad (2)$$

$$\frac{d^2\psi}{dy^2} = -\frac{\sum_i F c_i z_i}{\epsilon_0 \epsilon_f} \quad (3)$$

Where δ , F , ϵ_0 , ϵ_d , and ϵ_f are the dielectric thickness, Faraday constant, permittivity of free space, relative permittivity of the dielectric, and electrolyte respectively. Application of positive gate potential to a negatively charged surface (Fig. 1) increases the co-ion (negatively charged) concentration inside the nanochannel to satisfy Equation 1. Equation 2 indicates that the potential drops linearly across the dielectric from a magnitude of V_g at the gate to a constant surface potential of ψ_0 at the nanochannel wall. To produce a significant alteration to the potential distribution and ion concentration inside the nanochannel, $d\phi/dy$ must be non-trivial. This can be achieved by either increasing the applied gate potential or decreasing the dielectric thickness.

Figure 1: Schematic showing modulation of ion concentration inside nanochannel due to field-effect gating. Application of positive gate bias ($V_g > 0$) increases co-ion concentration and negative bias ($V_g < 0$) increases counter-ion concentration when compared to zero bias.

METHODS

Numerical Model

A coupled numerical model was solved in COMSOL Multiphysics (v5.2a) to calculate the electric-field and ion distribution inside the model gated nanofluidic device (Fig. 2). Poisson equation (electrostatics) was coupled with Nernst-Planck equation (Transport of diluted species) and Navier-Stokes equation (fluid flow) [8]. The surface charge density of the nanochannel and reservoir walls were assigned -5 mC/m^2 [9]. Specific gates were defined on top of the 40 nm thick dielectric. No-slip boundary conditions were defined for all walls. Reservoir protein concentration was fixed to 0.1 mM for the simulations, matching experimental condition. A custom meshing scheme was defined and refined, until mesh insensitive solutions were obtained.

Experimental Methodology

Our proof-of-concept nanofluidic field-effect chip dramatically advances our past work on fundamental investigation of ion transport with a “single gate” from Hilton Head 2014 and 2016 [10,

Figure 2: Geometry of the Reservoir-Nanochannel-Dielectric 'field-effect' gated system. Coupled Nernst-Planck, Poisson, and Navier-Stokes equation were solved in COMSOL Multiphysics (v5.2a) to estimate the spatially varying electric-field induced inside the nanochannel due to gating. Here, $\mathbf{J}_i$ is the species flux constituting the diffusion, electro-migration and convection components. For a given species i , D_i is the diffusion coefficient, ξ_i is the ionic mobility, z_i is the ion valence, $\mathbf{u}$ is the convective velocity. In addition, ρ_s is the volumetric space charge density, μ is the electrolyte viscosity and p , the pressure distribution inside the reservoir-nanochannel system.

11]. The device uses two microfluidic channels (8 μm (H) x 50 μm (W) x 3 cm (L)) as fluidic reservoirs for a bank of three nanochannels 16 nm deep (30 μm (W) x 2.5 mm (L)) [10, 11]. An overview of top cover fabrication with embedded individually addressable electrodes is given in Fig. 3. Each electrode is made of Chromium and is 10 nm high and 30 μm wide.

Figure 3: Fabrication sequence for embedding multiple gate electrodes on glass slide.

Several considerations were taken into account during the top cover fabrication. Chromium has a higher thermal conductivity compared to soda lime glass which helps to achieve adequate baking of photoresist AZ5214 during image reversal bake. Absence of Cr metal mask resulted in photoresist peeling during development. Second, a UV exposure of 3.5 secs and short development (35 sec) was chosen to have a straight side-wall profile of AZ5214. Having

a negative side-wall profile (typical during a negative PR development) causes insufficient dielectric coverage during dielectric sputtering, shorting the channel when a potential is applied. Third, Cr and SiO_2 were sputtered at the same batch, to ensure proper insulation of Cr. Adequate SiO_2 coverage over the Cr electrode was verified by immersing the top cover in a Chromium etchant solution, followed by piranha cleaning for 15 minutes. Fourth, to remove inconsistencies in the deposition rate while sputtering Cr and SiO_2 , deposition rate was calibrated before each batch. The fabricated top cover electrodes and the channel slide were then bonded using the Calcium bonding recipe [12]. The final schematic of the bonded nanofluidic channel, with the multi-electrode top cover is shown in Figure 4. The microfluidic and nanofluidic channels were initially filled with 0.1 mM Bovine Serum Albumin (66.5 kDa, stokes diameter = 6.96 nm) conjugated with Tetramethylrhodamine (TRITC, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 541/572 \text{ nm}$) in Phosphate-buffered saline (pH 7.4). Potential difference of 2 V (V_{gate}) was applied between gates 1–2 and gates 3–4 simultaneously (Fig. 4).

Figure 4: Schematic of bonded nanofluidic chip with inset showing AFM image of 16 nm dep nanochannel.

RESULTS

Universal gate Vs Local gate

Coupled multi-physics models using COMSOL Multiphysics showed that compared to a universal gate (Fig. 5a), application of gate potential in the present system (Fig. 5b) results in spatially varying non-uniform potential distribution in the gated and ungated region (Fig. 5c, d). In the case of a universal gate, no changes to the potential was observed inside the nanofluidic channel (Fig. 5a, c). Application of gate potential in the present system causes a linear drop in potential across the 40 nm thick dielectric (Fig. 5d). To satisfy Equation 1, the local space charge density inside the nanofluidic channels is altered. Gating therefore, introduces a non-linear gradient in potential distribution inducing a non-uniform electric field inside the nanochannel (Fig. 5c, d).

Figure 5: Design, potential distribution, and working principle of (a) 'universally' gated nanofluidic channel and (b) individually addressable multi-electrode nanofluidic chip (present work). (c) Representative plots showing local potential changes inside the nanofluidic channels in the present device between gates 1 and 2 compared to uniform potential (similar to flow by diffusion) in the 'universally' gated nanochannel. Voltage inside the nanochannel under no gating is normalized to a value of 1. Application of gate potential modifies volumetric space charge density inside the nanochannel to satisfy electro-neutrality, resulting in modified potential distribution compared to ungated scenario [3, 5]. (d) A non-linear (and non-uniform) electric field inside the nanochannel drives the charged proteins via electromigration for faster amplification. Electric fields of opposing polarity at the either ends of gate 1, facilitate amplification of BSA transport (Red arrows) inside the nanochannel. Inset shows potential distribution (in V) inside the 40 nm deep SiO₂ dielectric layer.

BSA Capture, Amplification, and Release

The non-uniform electric field was used to tune the pre-concentration of BSA at various segments of the nanochannel in a time-dependent manner (Fig. 6) for amplification (pre-concentration) and subsequent release of BSA. The e-field facilitates BSA capture and amplification via electromigration up to ~6x (Fig. 6b, e) the initial

concentration (Fig. 6a, e) in gates 2, 3 within 20 min. The depleted BSA at gates 1, 4 are subsequently replenished by a negative e-field on the far-side of the gates (Fig. 5c) that amplifies BSA concentration by ~6x, 9x at gates 1, 4 respectively. Upon removing V_{gate} , ~50% of trapped BSA is "released" driven by diffusion from all the gates within next 90 min (Fig. 6d, f). In the absence of applied gate potential, amplification or release was not observed during 120 min of imaging.

Figure 6: Fluorescence of BSA-TRITC inside the 16 nm deep channel was imaged when gates 1, 4 was set to a potential of +2 V while gates 2, 3 were grounded (exposure time ~ 30 s). Fluorescence images of BSA amplification at (a) $t = 0$ min, (b) $t = 20$ min and (c) $t = 30$ min. (d) Representative BSA "Release" phase fluorescence image at $t = 120$ min indicating reduction in BSA-TRITC concentration. Blue dashed line indicate nanochannel boundary in Fig. 6a and white-solid line indicates gated regions in Fig. 6a – d. (e) At $t = 15$ min, BSA concentration at gate 2 is ~2.5x higher than gate 3, as the shorter distance (30 μm) between gates 1 and 2, compared to 90 μm between gates 3 and 4 promote a higher local field to trap BSA at gate 2. At $t = 20$ min, BSA concentration saturates to 5.5x initial concentration at gates 2 and 3. (f) Switching off gate voltage at $t = 30$ min leads to gradual diffusion driven protein release over the next 90 min.

In summary, a novel system was demonstrated to trap and enrich protein concentrations using electrostatic field effect gating inside a nanofluidic chip with spatially inhomogeneous electric fields providing a new avenue for protein analysis.

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