Stable focusing of proteins within a packed microbead bed by ion concentration polarization in a paper-based analytical device

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Movie S1: 80 nM Texas-Red BSA enriching in a μb-ICP-PAD composed of 0.2 μm beads

Movie S2: 80 nM Texas-Red BSA enriching in a ICP-PAD without beads

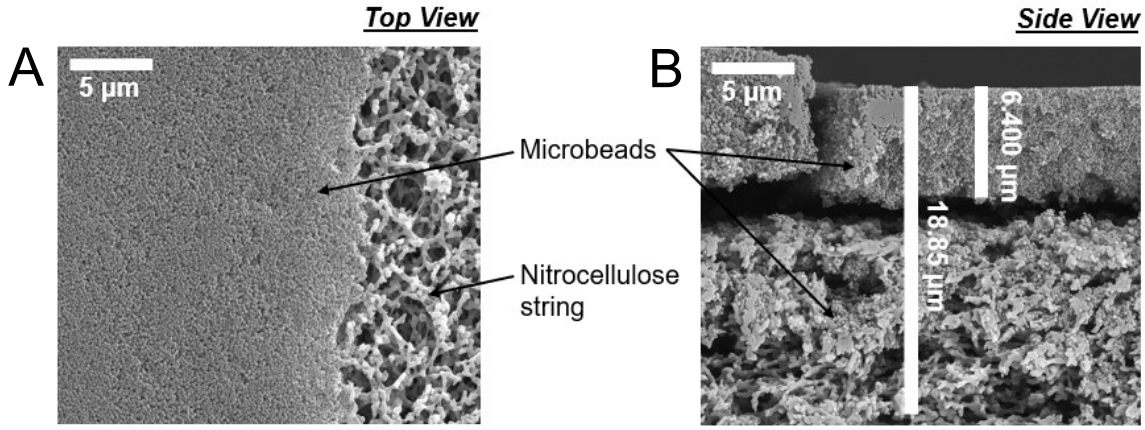

Fig. S1 SEM micrographs showing the (A) top view as well as (B) cross-sectional side of a packed microbead bed paper channel, revealing penetration of the nitrocellulose pores by the microbeads.

Fig. S2 Current-voltage curves recorded for Texas-Red BSA. A plot of current (μA) versus applied voltage (V) for three separate μb-ICP-PADs. Each trace represents one individual trial. The voltage was ramped from 0 to 70.0 V at a rate of 1.0 V per 10 s. The concentration of the fluorescent tracer was 80 nM. Solution composition consisted of 100 mM Tris HCl, 0.05% Tween-20, pH 8.2.

Experimental Details

Biotinylation of Texas Red BSA. The protocol for biotinylation followed manufacturer guidelines and recommendations for the Biotin Conjugation Kit (Fast, Type B), Lightening Link®. As this kit is designed for antibodies, which are more than twice the size of BSA, half the recommended starting amount was used to ensure no unlabeled proteins at the end of the reaction. 500 μg of Texas Red BSA was suspended in 50 mM Tris HCl at a concentration of 5 mg/mL. 100 μL of modifier was added to the protein solution and gently mixed using a pipet. This solution was then added to the lyophilized material for resuspension and incubated at room temperature for 15 min. After incubation, 100 μL of quencher was added to complete the conjugation.

Dialysis of the Biotinylated Protein. In order to remove any unbound biotin, the biotinylated Texas Red BSA sample solution was dialyzed using Slide-A-Lyzer Dialysis Cassettes (10K MWCO). A conical tube was filled with dialysis buffer (50 mM Tris HCl) and the sample was added to the device. The device was then slowly placed into the conical tube and constant contact was maintained between the membrane and dialysis buffer. The sample solution was then dialyzed on a shaker at 100 RPM for 2 hours, before exchanging with fresh dialysis buffer and dialyzed overnight. Finally, the sample was collected and stored at 4º C for further use. Sodium azide was added at a concentration of 0.05% (w/v) to prevent bacterial growth during storage.