Simultaneous purification and fractionation of nucleic acids and proteins from complex samples using bidirectional isotachophoresis

Supporting Information

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This document contains the following supplementary figures and information further describing our bidirectional ITP-based technique for simultaneous purification and fractionation of nucleic acids and proteins from complex biological samples:

DNA and protein ITP zones

We imaged focused DNA and protein zones during on-chip bidirectional ITP. For these visualization experiments, we labeled DNA with fluorescence dye SYTO64. Serum samples were spiked with salmon sperm DNA to enhance fluorescence signal. We spiked yellow fluorescent protein (YFP) to monitor protein ITP zone. Typical shapes and fluorescence signals of DNA and protein zones are shown in Figure S-1.

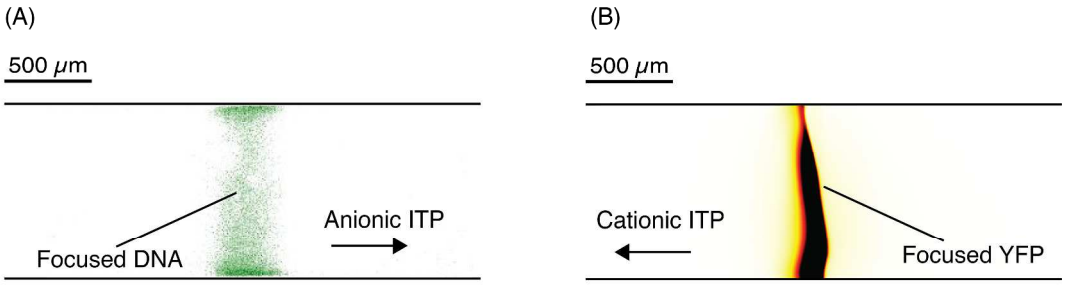

Figure S-1. Typical images of DNA zone and protein zone of human blood serum samples during bidirectional ITP: a) Fluorescent image of purified DNA ITP zone which migrates toward the anode in anionic channel; b) Fluorescent image of spiked YFP in the protein ITP zone. YFP and other extracted serum proteins migrate toward the cathode in cationic ITP channel.

Experimental set up

We monitored the ITP zone using epifluorescent microscopy (Figure S-2). We applied +10 μA constant current across the channel and recorded voltage over time using a sourcemeter (Keithley 2410, Cleveland, OH) interfaced with a computer running a custom Matlab (Mathworks, Inc) script. We performed on-chip visualizations using an inverted epifluorescence microscope (Nikon Eclipse TE300) (Nikon, Tokyo, Japan) equipped with a 4× objective (UPlanApo, NA 0.16; Nikon, Tokyo, Japan). A mercury lamp (Model C-SHG; Nikon, Tokyo, Japan) was used for excitation. We used filter cubes optimized for detection of YFP (XF105-2, Omega Optical, Inc., Brattleboro, Vermont) and SYTO 64, and a 0.63× demagnification lens (Diagnostic Instruments, Sterling Heights, MI). We captured images using a 1300 × 1030, 12-bit, interline CCD camera (MicroMAX-1300Y, Princeton Instruments, Trenton, NJ).

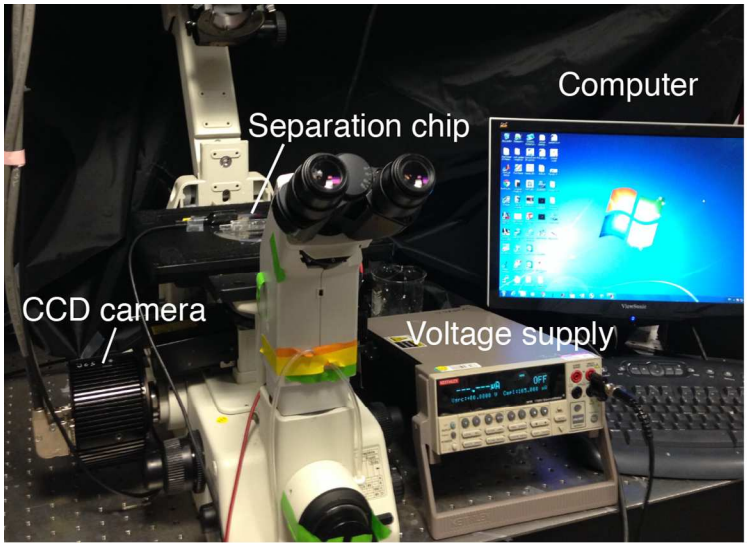

Figure S-2. Experimental setup. This set up includes an inverted epifluorescence microscope, a CCD camera, a voltage supply, and a computer for control of experiments and data acquisition.

Simulation and visualization of dynamics of bidirectional ITP

We simulated the dynamics of ITP zones in bidirectional ITP by using Stanford Public Release Electrophoretic Separation Solver (SPRESSO). In this simulation, we considered a finite sample zone injected into a straight channel as an initial condition. The simulation results are shown in Figure S-3(A). In this simulated spatiotemporal plot (time and distance along the channel in the ordinance and abscissa, respectively), the DNA and proteins focus into sharp counter migrating bands.

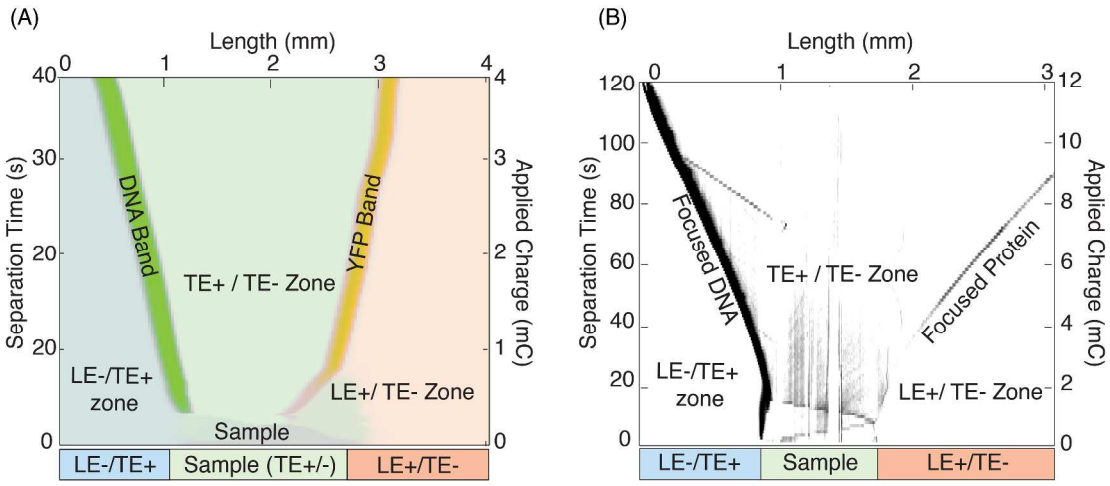

Figure S-3. Simulation and visualization of the dynamics of DNA and protein zones during bidirectional ITP process: A) Spatiotemporal representation of numerical simulation of ion concentrations during ITP. Each ion is given its own false color. B) Measured spatiotemporal plots determined from CCD images of protein and DNA zones focused in bidirectional ITP. Yellow fluorescent protein and DNA labeled with SYBR Green I were imaged using a custom fluorescence setup and a stereoscope.

We performed visualization experiments with the same system buffers described in the main paper but using a smaller version of the separation device with a straight (rather than S-shaped) channel configuration. This version of the device is depicted in Figure S-4. This device allowed us to simultaneously capture both the cationic and anionic ITP zones in the field of view of single CCD images. A resulting experimental spatiotemporal plot is shown in Figures S-3(B). As ITP proceeds, the DNA and protein ITP zones are clearly visible, each propagating in opposite directions away from the sample zone.

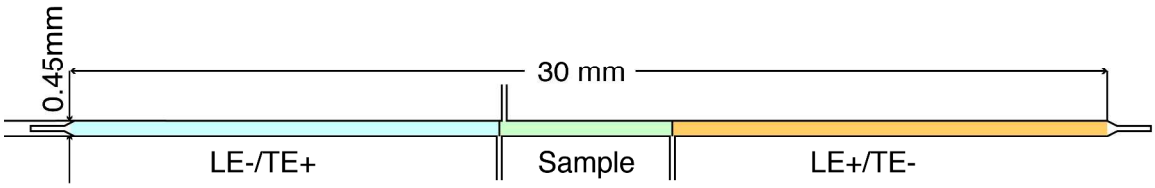

Figure S-4. Simple, straight channel design for visualization experiments. The main channel here was 0.45 mm wide and 30 mm long. Three side channels were used for loading buffers and samples. These also partition the main channel into three regions. We loaded samples to the middle region, LE-/TE+ buffer to the left region for DNA purification, and LE+/TE- buffer to the right region for protein extraction. The visualizations for this channel are shown in Figure S-3B.

Low fluorescence of DNA zones

In all experiments, we observed that ITP zones of DNA extracted from serum samples exhibited lower-than expected fluorescence (see Figure S-5). This was true even for cases where we spiked on the order of 20 ng of salmon sperm DNA into serum samples for visualization. We hypothesize that this weak fluorescence is due to nonspecific binding between DNA and serum proteins. This binding led to aggregates formation during sample preparation, thereby resulting in DNA loss.

We performed several experiments spiking DNA, YFP, or both DNA and YFP into pure buffer. As expected, spiking either DNA or YFP resulted in no observable aggregates. Spiking both DNA and YFP resulted in significant aggregates observable with the naked eye, which we attribute to DNA-protein complexes.

We hypothesize that DNA and protein complexation affected all of our experiments. However, despite this effect, we were able to extract sufficient DNA to detect endogenous copies of the BRAC2 gene.

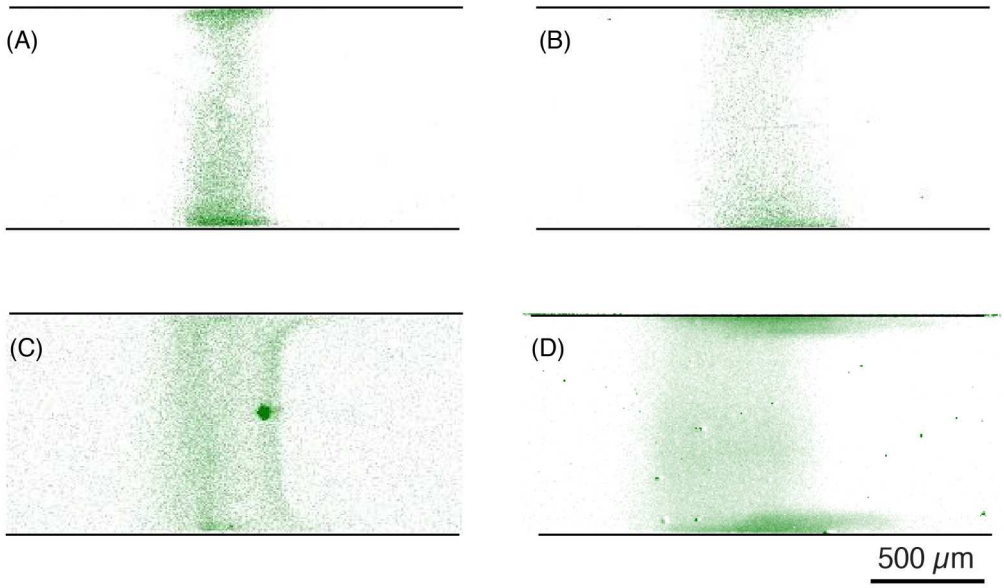

Figure S-5. Example images of fluorescence of DNA zones in ITP process from serum samples. (A)-(D) represent typical DNA zone shapes and fluorescence level in simultaneous extraction experiments where we spiked in salmon sperm DNA. We hypothesize nonspecific binding between DNA and serum proteins resulted in low collection efficiency for spiked DNA.