Supplementary information

Digital Microfluidics-Like Manipulation of Electrokinetically Preconcentrated Bioparticle Plugs in Continuous-Flow

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Movies

Movie S1: Time-evolution of the upstream and downstream transport of a single preconcentrated molecule plug (A) and splitting and merging of a single preconcentrated plug, corresponding to Fig. 2.

Movie S2: Time-evolution of formation of multiple plugs and transport to downstream locations in 4- and 6-membrane systems, corresponding to Fig. 3a and Fig. S2.

Movie S3: Time-evolution of generation of multiple preconcentrated plugs with continuous collection of the upstream preconcentrated molecules at the downstream plugs, corresponding to Fig. 3b.

Movie S4: Formation of multiple plugs and transport to downstream locations in various geometries (distances) of serial membranes.

Movie S5: Time-evolution of the manipulation of different locations of preconcentrated plug as by digitally controlled 8 serial membranes, corresponding to Fig. 5b.

Movie S6: Time-evolution of the manipulation of preconcentrated plugs comprised of two different biomolecules (GFP, RFP) introduced from different inlets in a 2D cross channel, corresponding to Fig.5c and d.

Movie S7: Time-evolution of the manipulation (merging/separation) of preconcentrated plugs comprised of various fluorescent biomolecules (GFP, RFP) in a 2D cross-channel, corresponding to Fig. S5.

Movie S8: Time-evolution of control (valving) of the preconcentrated plugs of red, green and blue fluorescent molecules, within a Y-shape microchannel with multiple inlets, corresponding to Fig. 6.

Movie S9: Time-evolution of fluorescent molecule sorting within a Y-shaped microchannel, in which the sorting direction was controlled by changing the polarity of the biased membrane (in red-dashed rectangle) and time-evolution of a combination of sorting and downstream preconcentration of the molecules (blue-dashed rectangle).

Supplementary figures

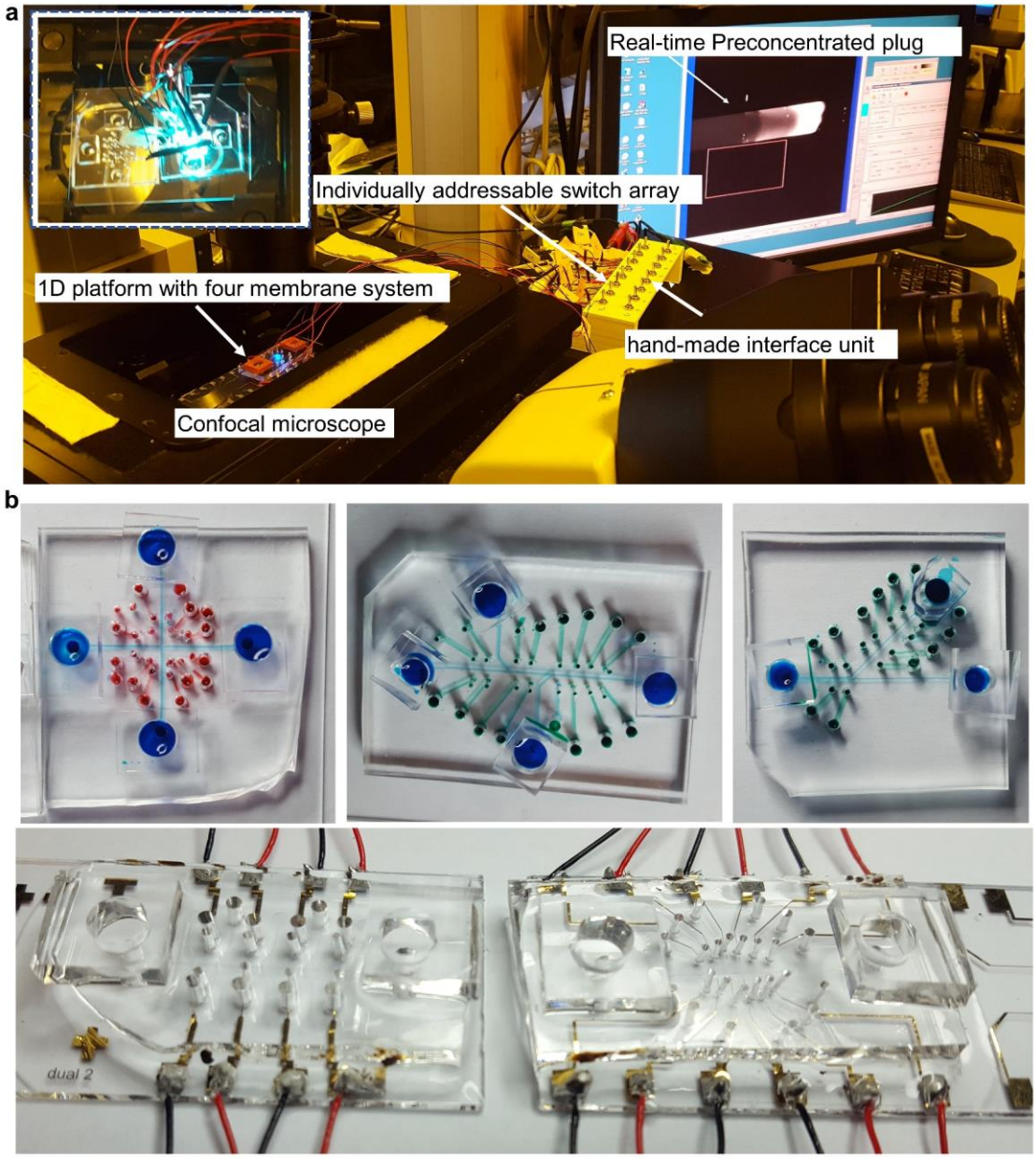

Fig. S1 (a) Experimental setup for digital control of a serial membrane system for actuation of the different membranes and (b) images of the different fabricated microfluidic platforms. The inset photo in (a) shows the external platinum electrodes introduced into a 2D channel geometry system.

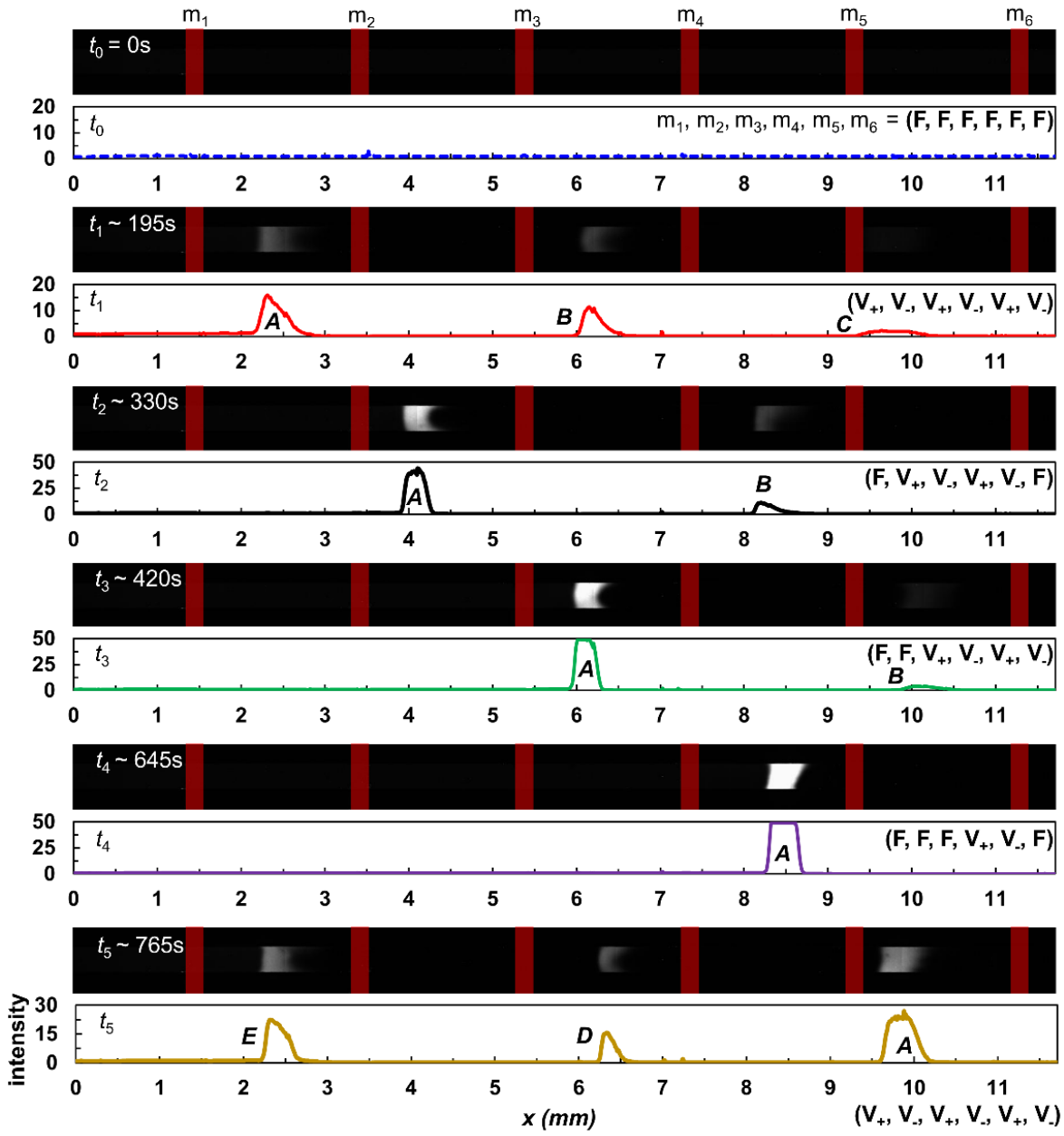

Fig. S2 Digitally controlled formation and downstream transport of multiple preconcentrated plugs within a 1D channel geometry with 6 embedded membranes. Similar to Fig.3a, the formed preconcentrated plugs (A, B, C) were transported downstream, under operating conditions of $\mu = 72 \pm 14$ µm s^{-1} (Pe ~ 796) from left to right and V= 15 V. The fluorescence intensity indicates the extent of Dylight fluorescent molecule preconcentration within a 23 µM KCl solution.

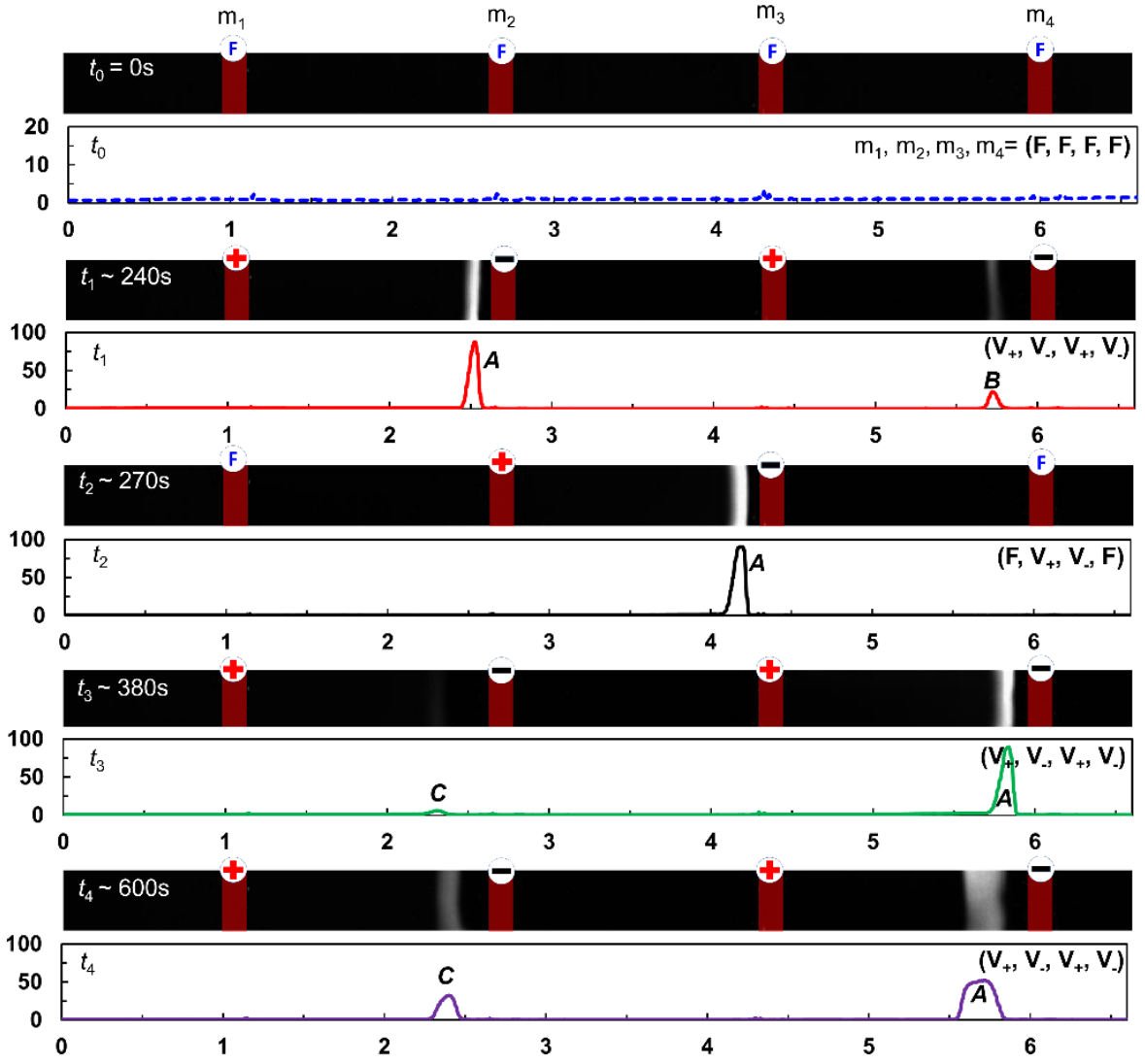

Fig. S3 Formation and downstream transport of multiple plugs (A, B, C) within a high-conductivity solution. The operating modes were taken from Fig. 3a, which periodically switched the operating mode between (V+, V-, V+, V-) and (F, V+, V-, F), under $\mu = 60 \pm 4$ μm s^{-1} (Pe ~ 669) from left to right and V= 30 V.

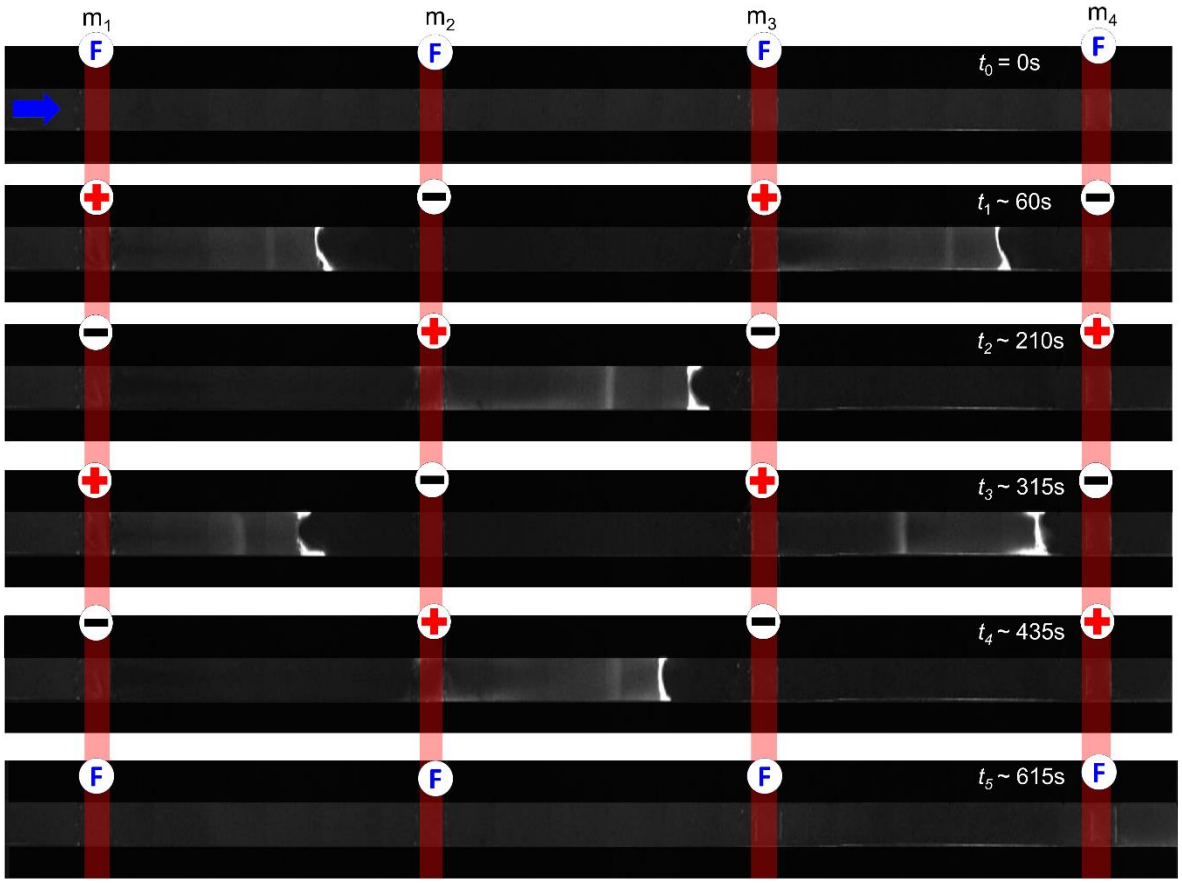

Fig. S4 Formation and downstream transport of multiple plugs of IgG1. Time-evolving fluorescence microscopy images of 10 µg mL^{-1} concentrations of fluorescent mouse IgG1 within a diluted high pH buffer (50 mM Tris, 1% bovine serum albumin, 0.05% Tween 20; pH = 9.4, conductivity = 80 µS cm^{-1}). The operating modes were taken from Figure 3a, which periodically switched the operating mode between (V+, V-, V+, V-) and (F, V+, V-, F), under average velocity of u ~ 30 µm s^{-1}, from left to right (blue arrow), and V= 25 V.

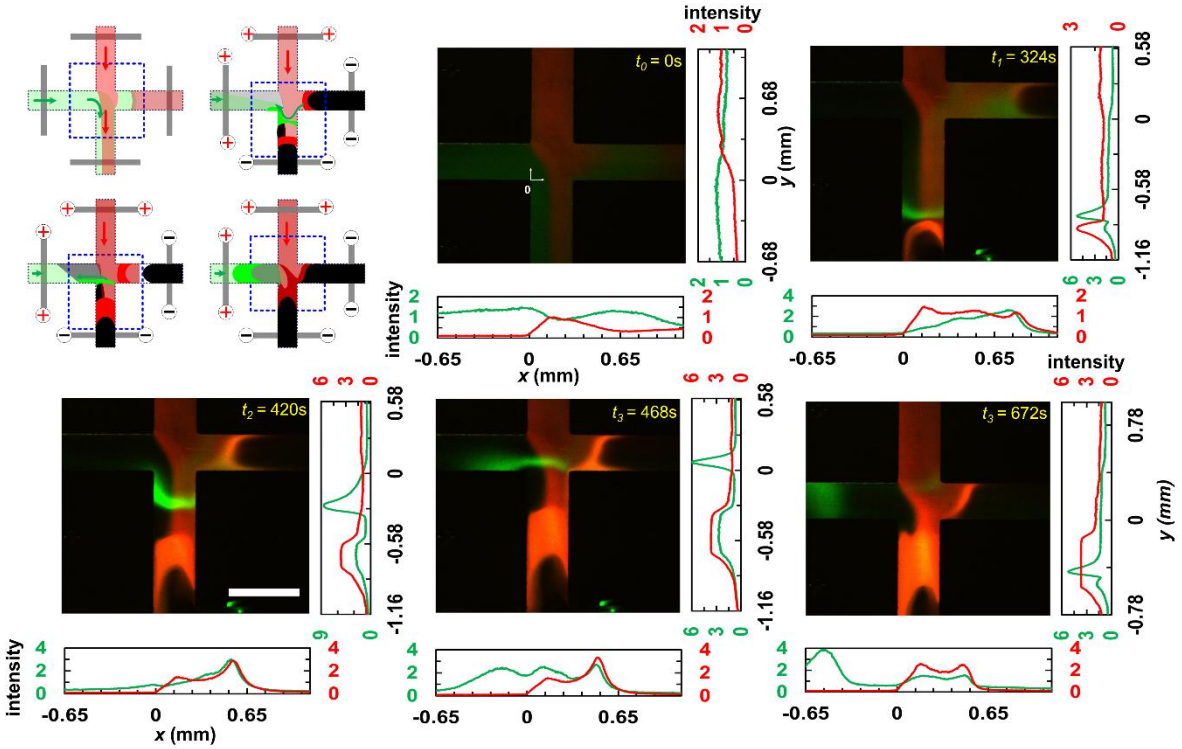

Fig. S5 Separation of mixed biomolecules (GFP, RFP) at the crossing of the horizontal and vertical channels within a 2D channel geometry. Horizontal and vertical depletion layers were generated between m_3 and m_7 using an operation mode of (F, V_+, V_-, F, F, V_+, V_-, F). The green and red lines in horizontal (x-axis) and vertical (y-axis) graphs indicate the corresponding normalized (with their initial intensity value) fluorescence intensity profiles of GFP and RFP, respectively. White scale bar: 0.5 mm.

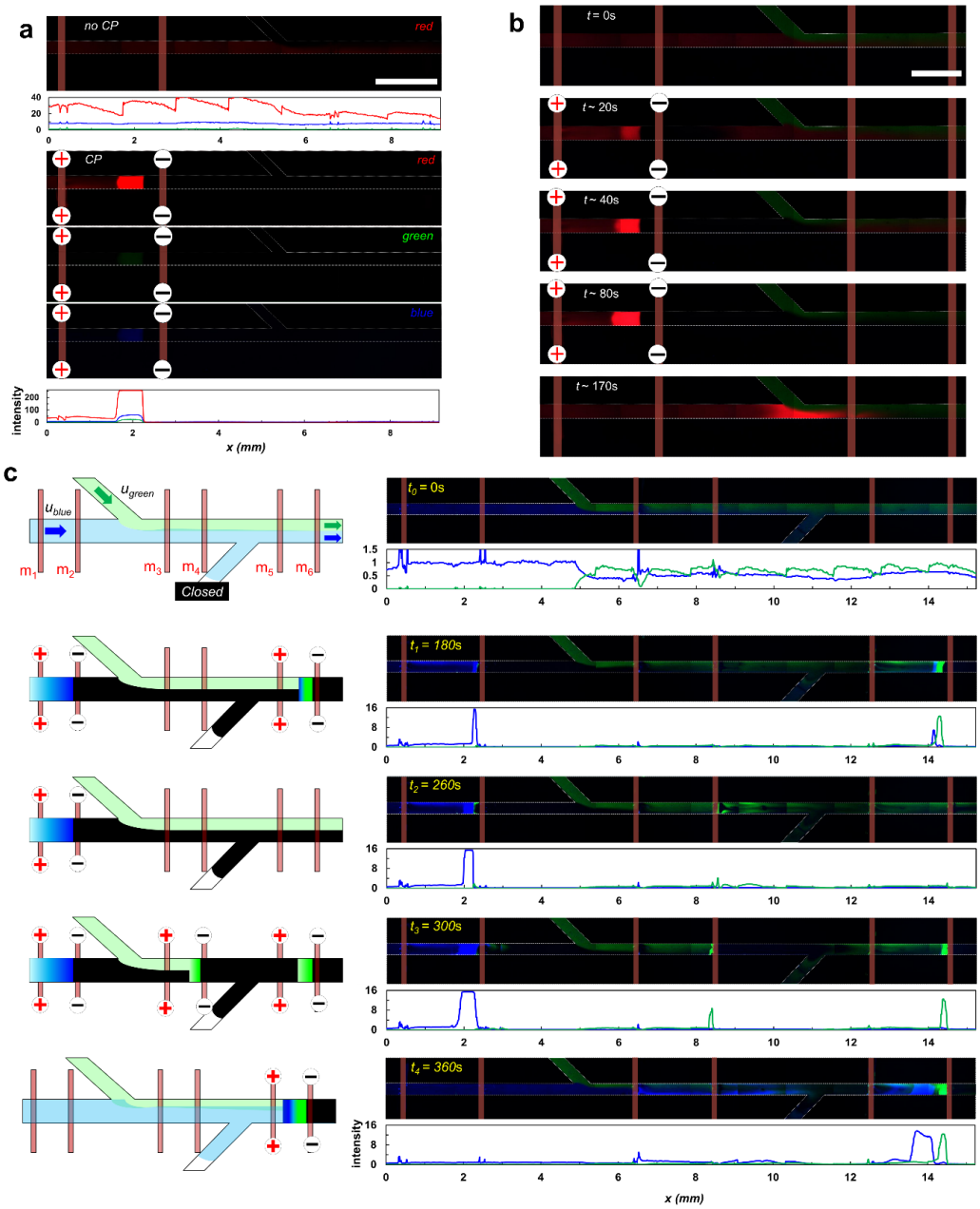

Fig. S6 Control (valving) of preconcentrated plugs of two species within a microchannel with a series of Y-shape junctions for multiple inlets. (a) Three fluorescence microscopy images of red fluorescent molecule (Dylight 594) in preconcentrated plugs during the CP using red, green and blue fluorescence filters and their overlapping intensities. (b) Time-evolving superimposed fluorescence (red and green channels) microscopy images showing valving, via a membrane pair located upstream to the Y-junction, of the red fluorescent molecules (Dylight 488) from the green fluorescent molecules (Dylight 594) that were introduced through different inlets. (c) Schematic description of time-evolving scenarios (left) along with superimposed fluorescence microscopy images showing blue and green fluorescent molecules (Dylight 405 and Dylight 488) (right) within a KCl solution (50 μS/cm) and corresponding normalized (with their initial intensity value) fluorescence intensity profiles along the main channel. The green and blue arrows in the schematics indicate the direction of flow and the blue and green lines in the graphs represent the normalized (by their initial intensity value) blue and green fluorescent intensities, respectively.

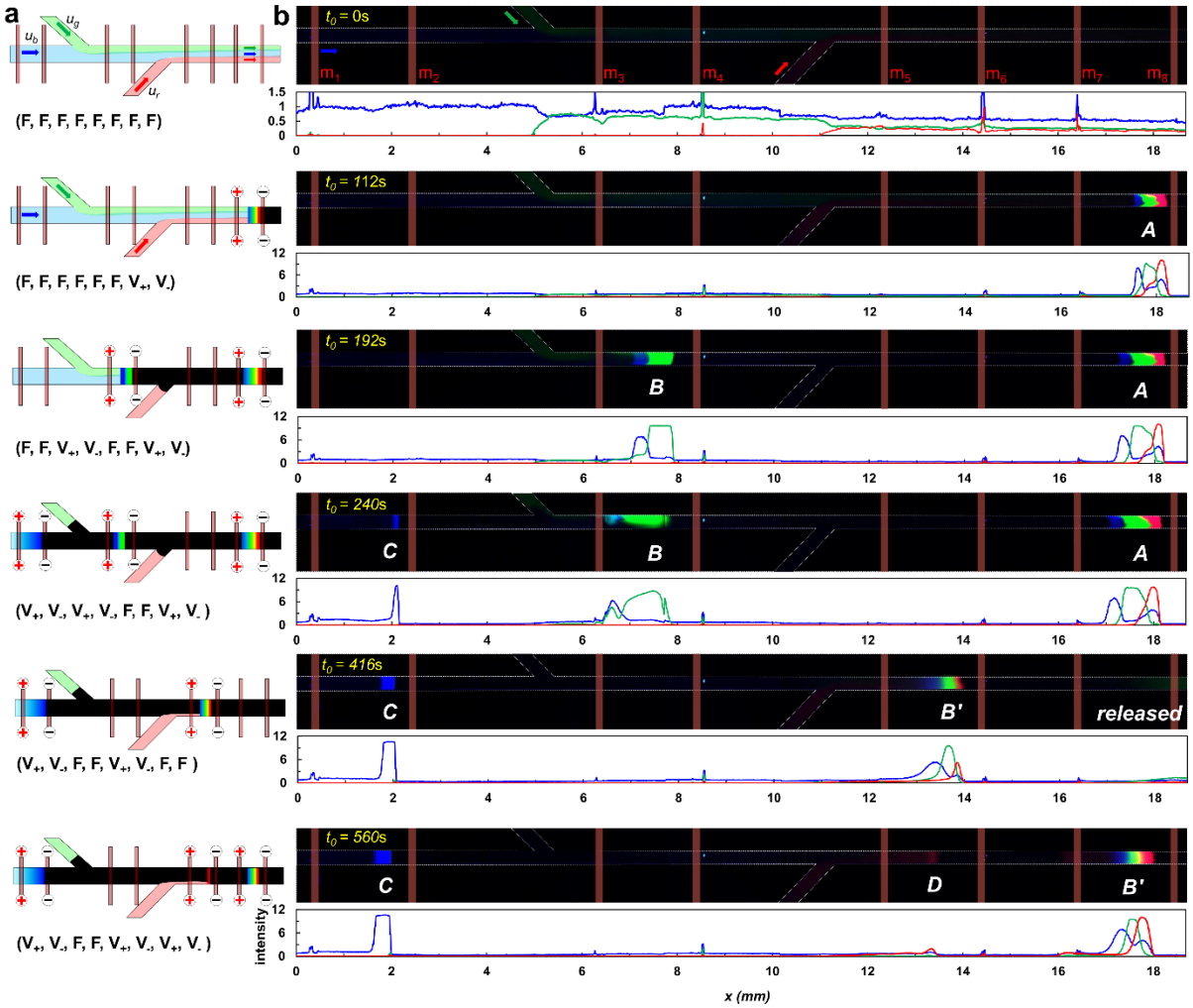

Fig. S7 Control (valving) of preconcentrated plugs of three species within a microchannel with a series of Y-shape junctions for multiple inlets. (a) Schematic of time-evolving scenarios (left) along with superimposed fluorescence microscopy images showing blue, green and red fluorescent molecules (Dylight 405, Dylight 488 and Dylight 594, respectively) (right) within a KCl solution (50 μS cm^{-1}) and corresponding normalized (with their initial intensity value) fluorescence intensity profiles along the main channel. The blue, green and red arrows in the schematics indicate the direction of flow and the blue, green and red lines in the graphs represent the normalized intensity of the blue, green and red fluorescence, respectively.

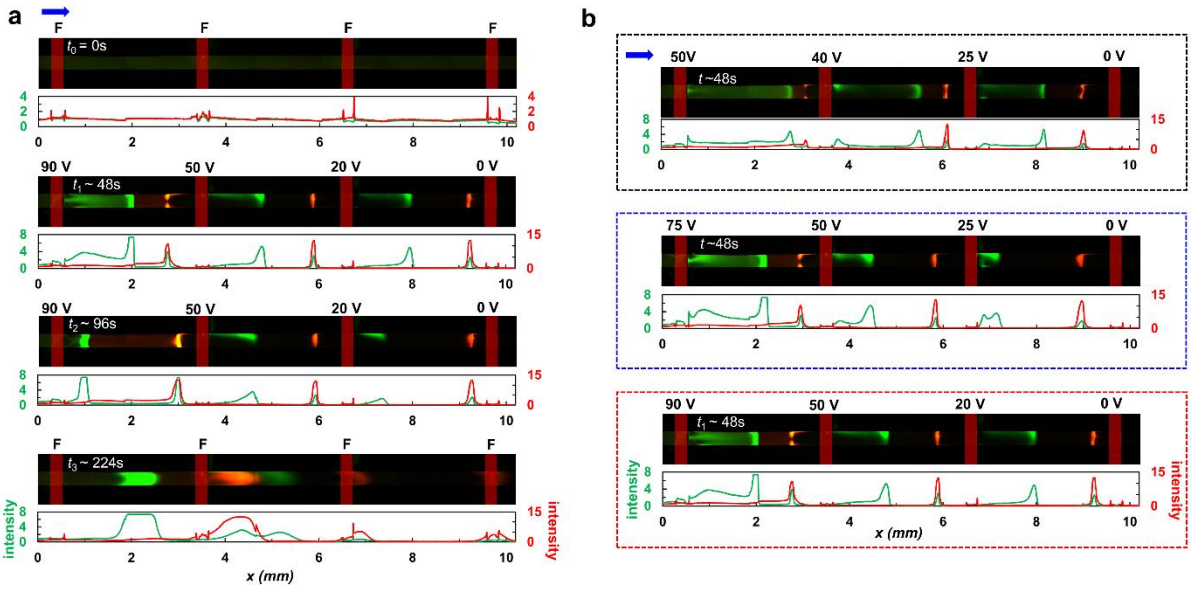

Fig. S8 Separation of preconcentrated plugs of two species under different voltages applied between pairs of membranes within a 1D channel geometry (a) Superimposed fluorescence microscopy images showing the separation of two fluorescent molecules (Dylight 488 and RFP) with different electrophoretic mobility manipulation, upon application of differential voltages on the four serial membranes (V_1, V_2, V_3, 0), and their corresponding green and red fluorescence intensity profiles. (b) Comparison of separation behavior under various voltage difference (ΔV) conditions simultaneously applied to the membranes (t = 48 s). The green and red lines in the graphs indicate the corresponding normalized (with their initial intensity value) fluorescence intensity profiles of Dylight 488 molecules and RFP, respectively.

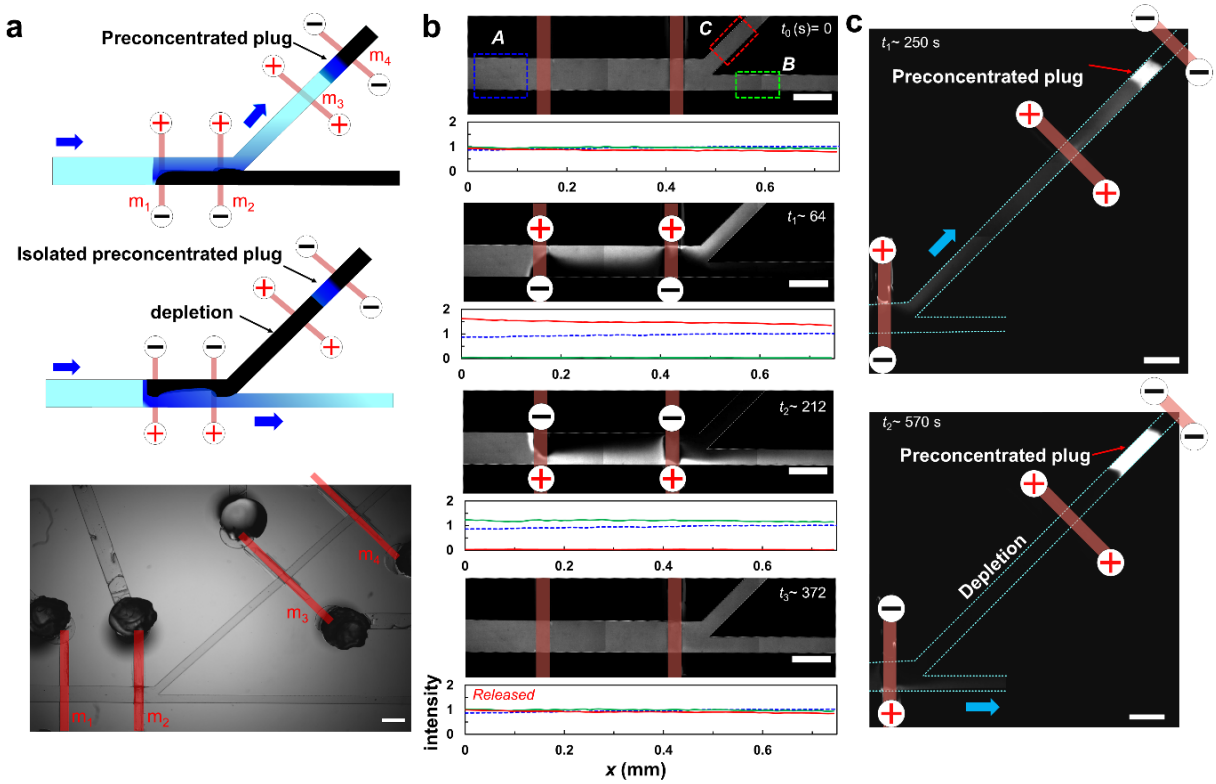

Fig. S9 Combination of the sorting and downstream preconcentration of molecules within a Y-shaped microchannel. (a) A schematic presentation of biosample sorting using a membrane in the upstream channel by opposite biasing (cathodic and anodic) at the end of the membrane located within the separated side chambers along with the microscopic images of fabricated chip. (b) Time-evolving fluorescence images showing the sorting of fluorescent molecules (Dylight 488 in within a KCl solution (50 µS cm^{-1})) at the Y-junction and corresponding normalized (by the average initial intensity value at region *A*) fluorescence intensity profiles. The sorting direction was controlled by changing the polarity of the biased membrane. The blue, green and red lines in the graphs represent the normalized intensity of the molecules at region *A*, *B* and *C*, respectively. (c) A representative scenario of combined sorting and preconcentration of the fluorescent molecules at a downstream channel. The operating CP conditions at b and c are V = 15 V and u ~ 247 ± 23 µm s^{-1}. White scale bar: 0.5 mm.