Microfluidic device for coupling isotachophoretic sample focusing with nanopore single-molecule sensing

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Supplementary Information

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SI-1: Co-focusing of tracking dye and labeled DNA

Unlabeled analytes may be conveniently tracked during ITP by introducing a fluorescent 'co-focusing' dye with matching electrophoretic mobility, so that both dye and analyte will be focused within the same migrating zone for label-free optical tracking. This is advantageous for avoiding labeling steps, which might anyway be ineffective when dealing with concentrations so small that even their focused zone would be difficult to optically detect. It is however necessary to ensure the absence of trace ions in the buffers with intermediate mobilities, which would focus between analyte and dye, creating a spatial offset – an ionic 'spacer'[1] – hence inaccurate delivery of analyte to the NP sensor.

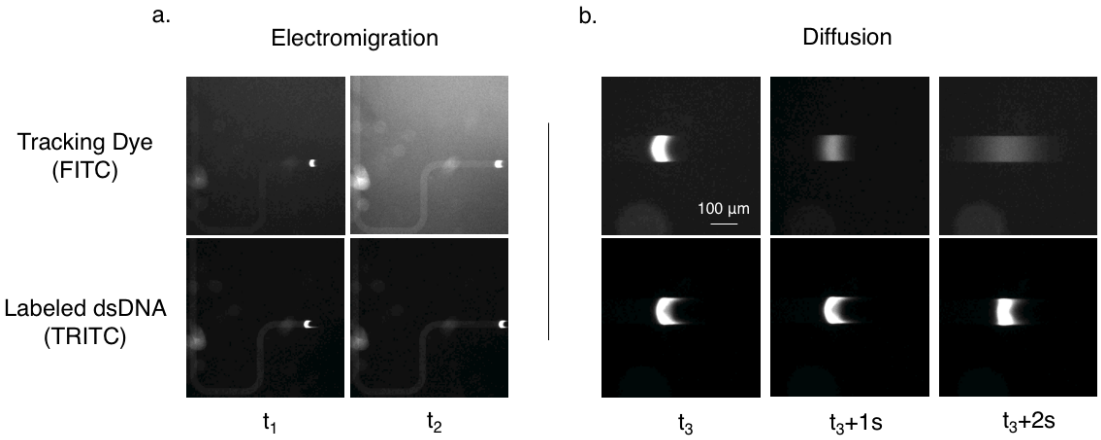

Figure S1. Co-focusing tracking dye and labelled DNA is monitored during (a) electromigration of the ITP plug and (b) during diffusion of the plug when the channel bias.

We qualify the absence of a significant spacer zone by performing ITP with a TE buffer containing 0.5 nM Dylight-488 and 1 pM ATTO-550-labelled 5kbp dsDNA, observable with FITC and TRITC filters respectively, to independently observe their positions during electromigration (time points t_1, t_2 t_3) and post-ITP diffusion (time points $t_3 + 1$ s, $t_3 + 2$ s). The fluorescent regions are seen to remain co-located. The Dylight-488 zone diffuses faster than that of the labeled dsDNA, as expected for a free dye vs. a polymer, yet the axial center position of the zones remains aligned.

SI-2: ITP calibration factor

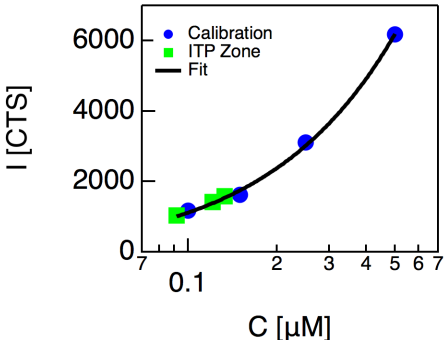

Figure S2: ITP calibration factor. An intensity-to-concentration calibration curve was created by filling the channel with known dye concentrations (0.1, 0.25, 0.5, 1.0 µM) (blue circles) and performing a linear fit (black line), in order to calculate the concentration of an ITP zone delivered to the nanopore (green squares). The analyte was unlabelled dsDNA (1 pM, 5054 bp) together with fluorescent tracer (100 pM Dylight 488).

SI-3: Pore diameter calculations

The nanopore diameter is inferred from the headstage measurements for open-pore current I under an applied bias V, via conductance $G = I/V$. Pore conductance is expressed as[2,3]

$$G = \left(\frac{4l}{\pi d^2} + \frac{1}{d}\right)^{-1} \sigma \tag{S.1}$$

for buffer conductivity σ, pore length l, and pore diameter d. Buffer conductivity is estimated from ionic composition using Peakmaster[4] and pore length according to ellipsometric measurements of the fabricated wafer's SiN_x layer to trivially solve (S.4) for diameter as

$$d = \frac{G}{2\sigma}\left(1 + \sqrt{1 + \frac{16\sigma l}{\pi G}}\right) \tag{S.2}$$

In the NP-only system, both *cis* and *trans* reservoirs were filled with LE buffer (600 mM KCl, 200 mM Tris, 175 mM HCl), σ = 7.6 S m^{-1}. Estimated pore diameter of 5.4 nm was calculated from equ. S.2 based on an open-pore current of 5.7 nA, and bias of 500 mV.

In the ITP-NP system, the conductivity within the pore was calculated based on the conductance of a 1:1 buffer mixture between *cis* and *trans* reservoirs. The *trans* reservoir was LE buffer and the *cis* buffer condition, at the location of the nanopore, was that of the freely diffusing ITP zone (Fig. 1c) – a 1:1 mixture of the LE and adjusted[5] TE buffers in the microfluidic channel. The adjusted TE ion composition was estimated using SPRESSO[6] as 220 mM KCl 180 mM Tris, 350 mM Tricine. From this we estimated σ_{TEadj} = 1.9 S m^{-1}, σ_{cis} = 4.8 S m^{-1} σ_{NP} = 6.2 S m^{-1} from Peakmaster. Finally, the estimated pore diameter of 6.4 nm was calculated from (2) based on as an open-pore current of 6.2 nA and bias of 500 mV.

SI-4: Events diagrams

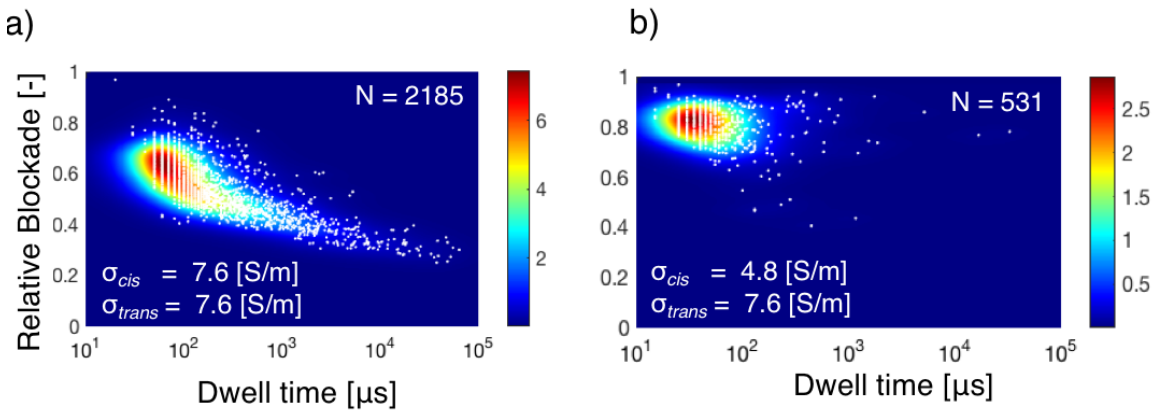

Figure S3. Scatter-plots of the events' dwell time and relative blockade depth (a) NP-only, and (b) ITP-NP. Colorbar corresponds to 2-D histrogram bin occupancy. In the NP-only case, both reservoirs were filled with LE buffer ($\sigma_{cis} = \sigma_{trans}$ = 7.6 S m^{-1}), and the *cis* reservoir contained a uniform concentration 1 nM dsDNA (5054 bp. The NP-only scatter-plot displays a hyperbolic curve, characteristic of pores translocating a mixture of folded and unfolded DNA wherein the "event charge deficit" of each translocation is a conserved quantity.[7] In the ITP-NP case, the sample reservoir contained a uniform 1 pM concentration of DNA focused to the position of the NP membrane within the microfluidic channel aperture via ITP. The local buffer composition of this local *cis* region is a mixture of LE and adjusted TE buffer (1:1 mixture of LE and TE-adjusted buffer is σ_{cis} = 4.8 S m^{-1}); the *trans* is filled with TE buffer (σ_{trans} = 7.6 S m^{-1}).

SI-5: Event-rate scaling

Diffusion-limited capture rate is a function of both local analyte concentration and pore geometry effects.[8] We therefore rescale event rates of the ITP-NP and NP-only system to more directly compare the relative gain in events per unit time by pre-concentrating analyte to the pore with ITP. The capture rate (events per unit time) can be estimated as proportional to the local analyte concentration *c*,

$$J = Rc, \tag{S.3}$$

where *R* is a function of pore geometry (length *l*, diameter *d*), analyte electrophoretic mobility μ, and NP voltage bias.

$$R = \frac{\pi d^2 \mu}{4l} V. \tag{S.4}$$

Performance of a given configuration due to changes in pore diameter is thus estimated as

$$J_2/J_1 = (d_2/d_1)^2. \tag{S.5}$$

Using the values in SI-3, the ITP-NP and NP-only scaling is $J_{ITP-NP}/J_{NP-Only} = \left(\frac{6.4}{5.4}\right)^2 = 1.4$. The corrected ITP-NP improvement in event rate per nanomolar concentration of analyte, as compared to an NP-only chip is then (2.56/1 pM)/(5.43/1 nM) / 1.4 = 337-fold.

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