Supporting Information

Coupled isotachophoretic preconcentration and electrophoretic separation using bidirectional isotachophoresis

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- In Fig. S1, we present further details on the injection protocol of bidirectional isotachophoresis (ITP) experiments (Fig. 3 of the main paper) for visualization of interacting anionic and cationic ITP shocks.

- In Sec. S2, we present a strategy of choosing electrolytes to couple ITP preconcentration and electrophoretic separation of weakly acidic species, such as amino acids. We describe in detail the choice of electrolyte chemistry and show a simulation of preconcentration and separation of two amino acids (cysteine and serine).

- In Fig. S3, we show a plot of resolution vs. time for bidirectional ITP and t-ITP simulations presented in Sec. 2.3 of the main paper.

S1. Protocol for visualization of shock interaction in bidirectional ITP

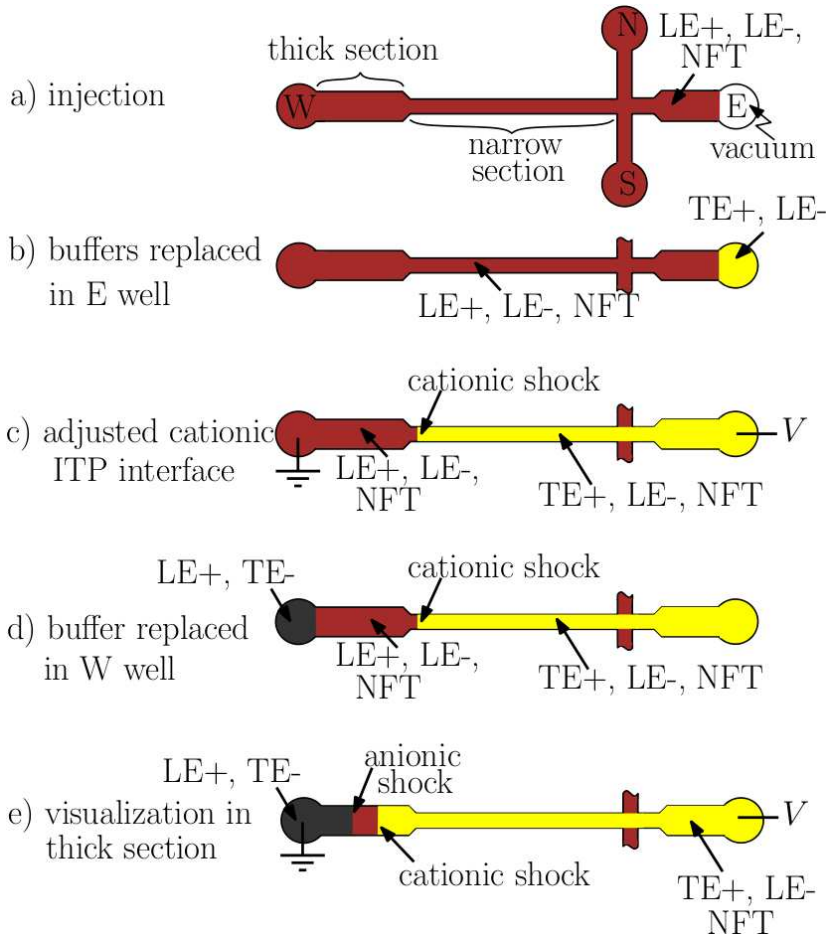

Figure S1: Protocol for visualization of interaction of anionic and cationic ITP shocks in bidirectional ITP. For visualization experiments on Caliper NS-95 chips: (a) we injected the mixture of LE+, LE- and non-focusing tracer (NFT) by applying vacuum on E well. We then emptied the E well and (b) filled the E well with TE+/LE- mixture. (c) We then moved the cationic ITP (LE+/TE+) interface by applying voltage between E and W wells. We performed this step to ensure that the anionic (LE-/TE-) and cationic (LE+/TE+) shocks interacted within the thicker cross-section channel. (d) We then emptied the W well and filled it with LE+/TE- mixture. (e) We then applied voltage between E and W wells and imaged the LE-/TE- and LE+/TE+ shocks in the thicker cross-section channel. This protocol was used simply to precisely time and place the shocks for quantitative visualizations of the shock interaction. We stress that setting up converging bidirectional ITP can be set up with simpler protocols (e.g., the protocol depicted in Figure 1 of the main paper).

S2.1 Method for preconcentrating and separating weak acids using bidirectional ITP

In Sec 2.1 of the manuscript we described a strategy for choosing electrolytes to couple ITP preconcentration and CE separation for the case of strongly ionized samples, such as nucleic acids. Here we describe the choice of buffers for weakly acidic analytes. For such analytes, we pursue a strategy wherein shock interaction decreases local pH of the anionic ITP zones, causing the effective mobility of analyte ions to drop below that of the TE- ions and thereby initiating electrophoretic separation. To this end, we can choose a relatively high pK_a base with high mobility as LE+ and a low mobility, weaker base as TE+. This creates a pH gradient across the cationic ITP shock, with a lower pH on the TE+ side. For the anionic ITP, we choose from relatively strong acids for LE-. We then choose a TE- with a lower pK_a than that of the analytes, but which also has low mobility. The latter is a key choice as we will use the shock interaction to titrate the anionic ITP zones to a lower pH, at which effective mobility of analytes decreases significantly compared to TE- ions, causing them to become slower than TE- ions.

Before the shock interaction, the pH of anionic ITP zones is high as the LE+ (a cation of weak base with relatively high pK_a) serves as the counter-ion. At high pH, weakly acidic analytes have high effective mobility and therefore focus ahead of slower TE- ions. When the cationic ITP shock interacts with the anionic ITP shock, TE+ (cation of relatively weak base) replaces LE+ (cation of a stronger base) as the counter-ion for anionic ITP. This decreases the local pH of anionic ITP zones and therefore decreases the local value of effective mobility of analyte ions. Whereas, the mobility of TE- ions does not decrease appreciably compared to analytes, as TE- is an anion of a stronger acid. If we make the choices of electrolytes correctly, the shock interaction causes the effective mobility of analyte anions to decreases to a value smaller than that of TE-. This then violates the ITP focusing conditions of analyte ions and initiates electrophoretic separation.

This scheme for preconcentrating and separating weakly acidic species differs from that for strongly acidic species in several aspects. Firstly, for weakly acidic species we use cationic ITP to titrate anionic ITP zones to a higher pH before the shock interaction and to a lower pH afterwards. This is in contrast to the scheme for strongly ionized anionic species, wherein the pH of anionic ITP zones increases after the shock interaction. Secondly, in the current scheme, electrophoretic separation occurs because the *mobility of analyte ions decreases below the mobility of TE- ions* after the shock interaction, while the mobility of TE- ions does not change appreciably. In contrast, in the case of strongly ionized anionic analytes, electrophoretic separation occurs because *the mobility of TE- ions increases above that of the analytes* after the shock interaction, but the mobility of analyte ions does not change appreciably.

We provide specific examples of viable electrolyte chemistries for extending our technique to anions of weak acids. Note that a key requirement is that LE+ should be a cation of a relatively stronger base with a pK_a higher than that of the TE+ and with a high fully-ionized mobility. While TE+ should be a cation of a weaker base with low fully-ionized mobility. Typically stronger bases have relatively high fully-ionized mobility compared to weak bases. Table 1 shows three choices each for cationic LE and TE (nine usable combinations of LE+ and TE+) which satisfy our requirements. Another requirement is that the effective mobility of analyte ions should be more than that of TE- ions when the buffering counter-ion is LE+ and otherwise when the counter-ion is TE+. In order to effect a substantial increase in the effective mobility of analyte ions after the shock interaction, TE+ and LE+ should be chosen such that

$pK_{a,TE+} < pK_{a,S-} < pK_{a,LE+}$. On the other hand, TE- should belong to an acid with a pK_a lower than that of the analyte ions, so that the mobility of TE- ions does not change appreciably, after the shock interaction, compared to the mobility of analyte ions. For example, in our simulations shown below in Figure S2, we used Hepes ($pK_{a,TE-} = 7.5$) as TE-, Ethanonamine ($pK_{a,LE+} = 9.5$) as LE+, Bistris ($pK_{a,TE+} = 6.4$) as TE+ and Serine ($pK_{a,-1} = 9.3,\ pK_{a,+1} = 2.2$) as one of the analytes. Lastly, the choice LE- is straight forward, and any fast anion will serve such as Cl^- or SO_4^{2-}.

Table S1: Possible cationic buffer systems for coupled preconcentration and separation of weakly acidic anions using bidirectional ITP.

cationic LE (LE+)	$\mu_{+1}\left(\times 10^{-9}\ \mathrm{m^2.V^{-1}.s^{-1}}\right)$	$pK_{a,+1}$
Ethanolamine	44.3	9.5
Ammonium	76.2	9.25
Amediol	33.5	8.78
cationic TE (TE+)	$\mu_{+1}\left(\times 10^{-9}\ \mathrm{m^2.V^{-1}.s^{-1}}\right)$	$pK_{a,+1}$
Bistris	26	6.4
Pyridine	30	5.18
Creatinine	37.2	4.83

S2.2 Simulation of coupled preconcentration and separation of amino acids using bidirectional ITP

To demonstrate the applicability of our technique for weakly acidic analyte species we performed simulations of coupled ITP focusing and electrophoretic separation of two amino acids (Cysteine and Serine) using bidirectional ITP. For our simulation we used the SPRESSO simulation tool [1,2] to solve one-dimensional species transport equations. We used 75 mM HCl as LE-, 20 mM Hepes as TE-, 150 mM Ethanolamine as LE+ and 150 mM Bistris as TE+. Initialy two analytes, Cysteine (S1-) and Serine (S2-), are mixed with the LE-/LE+ mixture each at a concentration of 80 µM. Figures S2a-b show the initial conditions of the simulation. When electric field is applied, LE-/TE- and LE+/TE+ shocks propagate towards the right and the left, respectively. Prior to shock interaction, S1- and S2- focus between the LE- and TE- ions, as shown in Fig. S2d. When the LE+/TE+ and LE-/TE- shocks interact (Figs. S2e-f) the effective mobility of Cysteine and Serine ions decreases below the mobility of TE- ions. This initiates electrophoretic separation of the analyte ions. Figures S2g-h show the final state where both analyte ions, S1- and S2-, are fully separated. We note that, for electrophoretic separation to occur it is necessary for TE- ions to overtake the focused analytes. However, TE- ions need not overtake LE- ions and the LE-/TE- shock may persist, as shown in Figs. S2g.

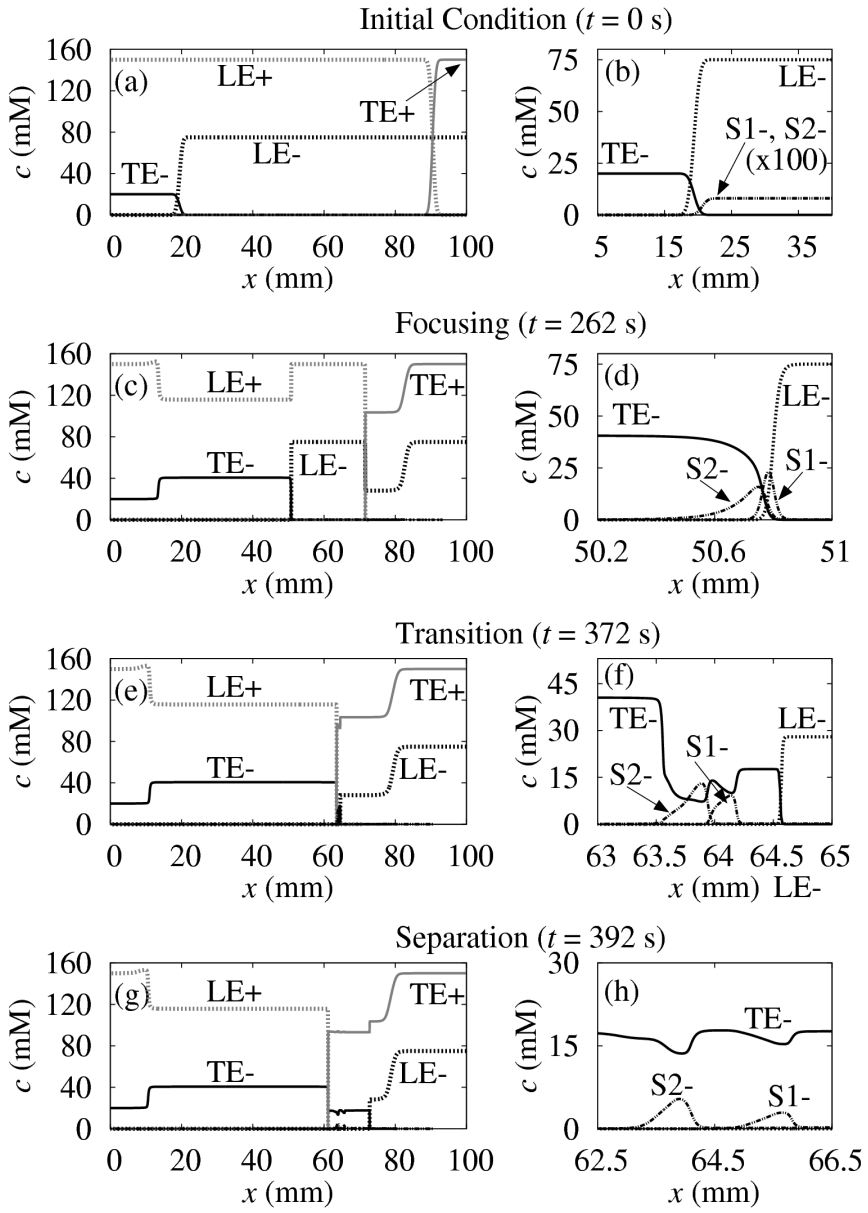

Figure S2: Simulation showing focusing and separation of two amino acids (Cysteine and Serine) using bidirectional ITP. Plots in second column are detailed views of the distributions in the first column. (a-b) show the initial distribution of chemical species in the separation channel, prior to activating current. (c-d) show LE-/TE- and LE+/TE+ shocks after the electric field is applied. (c) shows an LE-/TE- shock (x=50.5 mm) propagating rightward and a LE+/TE+ shock (x=70 mm) propagating leftward. (d) shows anionic analytes Cysteine (S1-) and Serine (S2-) focused between LE- and TE-. (e-f) show the transition from focusing to separation upon the interaction of LE-/TE-and LE+/TE+ ITP shocks. The low pH TE+ zone washes over the focused anionic analytes, decreasing the effective mobility of S1- and S2-, while only negligibly affecting the mobility of TE-, which is a stronger acid. Here, the effective mobility of S1- and S2- decreases below that of TE-, thereby initiating separation. (f) shows TE- overtaking focused S1- and S2-, thus initiating electrophoretic separation. (g-h) show the final state, in which analytes S1- and S2- are fully separated. (g) shows an anionic ITP shock at x=70 mm and a cationic ITP shock at

x=60 mm, after the shock interaction. (h) shows fully separated peaks of S1- and S2- in the CE mode. Simulations were performed using our open source code Spresso [1,2]. Chemistry is described in text. We assumed a constant current of 1.4 µA, and a D-shaped, wet-etched channel 74 µm wide and 12 µm deep. We approximately account for electroosmotic flow using a constant and uniform electroosmotic mobility of 2×10^{-9} m^2Vs^{-1}.

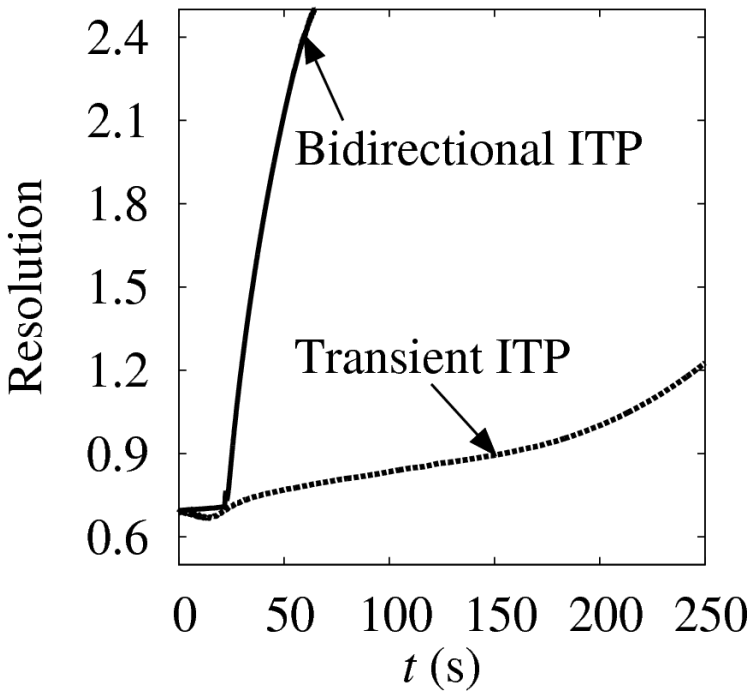

Figure S3: Simulations comparing separation resolution of transient ITP (t-ITP) and bidirectional ITP. The plot shows separation resolution versus time, *t*, for the simulations shown in Fig. 4 of the main paper. Here, we use the definition of resolution given by Giddings,[3] $\Delta L/\left(2\sigma_1+2\sigma_2\right)$, where ΔL is the distance between the two peaks and, σ_1 and σ_2 are the standard deviations of the corresponding peaks. For t < 25 s, both t-ITP and bidirectional ITP are in the focusing mode and therefore the resolution does not change considerably with time. Around t = 25 s, in both t-ITP and bidirectional ITP simulations, ITP preconcentration transitions to electrophoretic separation. Thereafter, the analyte peaks separate and the resolution increases monotonically with time. Comparison of the two curves shows that, for a given assay time, bidirectional ITP yields much higher resolution than t-ITP. That is, for a fixed distance between the two analyte peaks, the peaks in bidirectional ITP are much less dispersed than in t-ITP. Alternatively the plot shows that, to achieve a given separation resolution bidirectional ITP requires significantly less time and less channel length than t-ITP. For example, in the conditions we explore here, bidirectional ITP yields separation resolution of 1.2 just 10 s after the interaction of anionic and cationic ITP shocks. In contrast, similar resolution is achieved by t-ITP after 225 s from the initiation of CE separation. The simulation parameters and the electrolyte chemistry are described in Sec 2.3 of the main paper.

References

1. Bercovici, M.; Lele, S.K.; Santiago, J.G. *J. Chromatogr. A* **2009**, *1216*, 1008-1018.
2. Bercovici, M.; Lele, S.K.; Santiago, J.G. *J. Chromatogr. A* **2010**, *1217*, 588-599.
3. Giddings; J.C., *Sep. Sci.* **1969,** *4,* 181-189.