Supplemental Material for Emrich CA, Medintz IL, Chu WK, and Mathies RA, "Microfabricated Two-Dimensional Electrophoresis Device for Differential Protein Analysis", *Analytical Chemistry* (2007).

Incompatibility of the anionic detergent SDS with the microDIGE analyzer

The peak capacity of any 2-dimensional separation is the product of the peak capacities of the individual separation dimensions, but only if the separation mechanisms in each dimension are completely orthogonal. Most often, IEF and SDS-gel electrophoresis are use to separate proteins by pI and mass respectively, which are two such orthogonal properties. In slab gel 2DE systems, the dimensions are run separately and sequentially, with an equilibration step in the interim. This equilibation step prepares focused proteins for SDS-gel electrophoresis through the immersion of the IEF gel in a buffer containing SDS and other solubilizing agents.

In a constrained microfluidic system where the separation systems are directly connected, this equilibration step is not feasible and denaturing focused proteins is much more complicated. The design of the microDIGE analyzer, particularly MFI structures, was specifically aimed at this problem. By providing a method to discontinuously load two gel systems, the 2nd-dimension separation channels can be loaded with a gel containing SDS, facilitating the denaturing of focused proteins.

Li, et al. proposed a method for on-column denaturation of proteins during a 2DE separation in a microfluidic device, but we were unable to reproduce their results. Figure S1 presents the results of 2nd-dimension electrophoretic separations of a low-mass protein ladder (Dalt 7L, Sigma, minimally labeled with FITC) without first performing IEF. To elucidate the effects of SDS on protein separations, electrophoresis was carried out through 5% PDMA in 75 mM Tris-HCl, pH 8.7, at a field strength of 200 V/cm, with and without both SDS and heat treatment. Native electrophoresis (Fig. S1A) results in narrow peaks with excellent lane-to-lane reproducibility. Upon addition of 0.1% SDS to the run buffer (Fig. S1B), the lane-to-lane variation of retention time increases, as do the overall retention times. More significantly, a great fraction of the proteins are still contained in "front" and "rear" peaks that may be the boundaries of an isotachophoretic zone. Heat denaturation (Fig S1C) was attempted by pausing the separation 20 s after it commenced

heating the chip and holding at 85°C for 7.5 m, then resuming the run at the raised temperature. This approach significantly reduced lane-to-lane variation, but the front and rear peaks persist, obscuring all but the first three of the seven peaks in this ladder.

Denaturation temperatures from 35°C to 85°C and hold times from 0–7.5 minutes were investigated, and while increasing temperatures significantly reduce the lane-to-lane variation, they offer no solution to the front and rear peaks. For these reasons, subsequent experiments were carried using native gel electrophoresis for the 2nd-dimension separation. We are confident that with further investigation, particularly with respect to choosing appropriate electrophoresis buffers, this limitation can be overcome.

Reproducibility of 2DE of complex protein mixtures

One of the greatest challenges in developing and optimizing a microdevice for 2D electrophoresis is finding run conditions that yield repeatable run-to-run results. Early 2DE experiments on purified proteins nearly always resulted in reproducible peak elution patterns. However, upon transitioning to complex protein samples derived from cell lysates, peak elution patterns became irreproducible from run-to-run. This irreproducibility is exemplified in Figure S2 (A–D), which presents the results of four successive 2DE runs.

Reproducible separations of complex protein mixtures were achieved by lowering the 2nd-dimension electric field strength to 150 V/cm and by the inclusion of 7 M urea in the 2nd-dimension separation matrix. The results of four successive separations using these ideal run conditions are presented in Figure S2 (E–F), demonstrating excellent run-to-run reproducibility.

The underlying cause of run-to-run irreproducibility is unknown but may be due to electroosmotic flow induced by the nonspecific adsorption of protein to the walls of the 2nd-dimension channels. The addition of urea, therefore, may help to solubilize otherwise-adsorbant proteins and suppress electroosmotic flow. The irreproducibility in elution patterns is also evident in the electrophoretic current measured during the runs presented in Figure S2, which also exhibits significant run-to-run variation (Fig. S3A). Upon

switching to ideal run conditions, the 2nd-dimension run current becomes reproducible (Fig. S3B), perfectly mirroring the reproducibility of peak elution patterns.

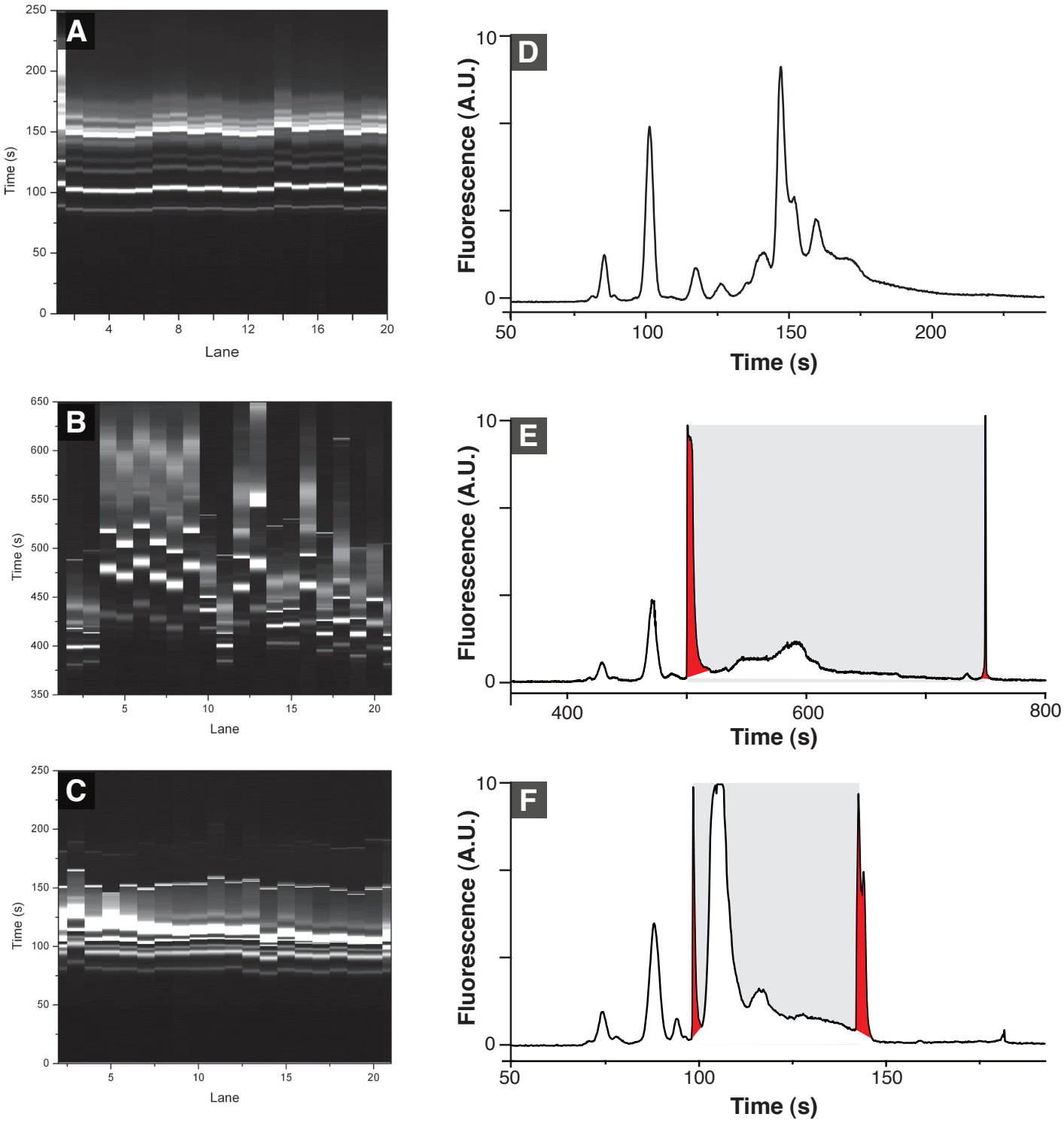
A comparison of variation in 2nd-dimension electrophoretic currents under different run conditions is presented in Figure S2C, showing the effects of changing the electric field strength and presence of urea. Electrophoretic current ranges are plotted as standard deviation about mean current. Under ideal conditions, the variation in current is minimal and the current remains relatively constant throughout the duration of the run. (Figure S3C, solid black trace, N=10). Performing the 2nd dimension separation without urea results in much wider current ranges, both at 150 and 300 V/cm (N=4 and 10, respectively). Using urea and the higher, 300 V/cm separation field resulted in irreproducible elution patterns, and a narrow, but falling current range (N=4).

Supplemental Figure Legends

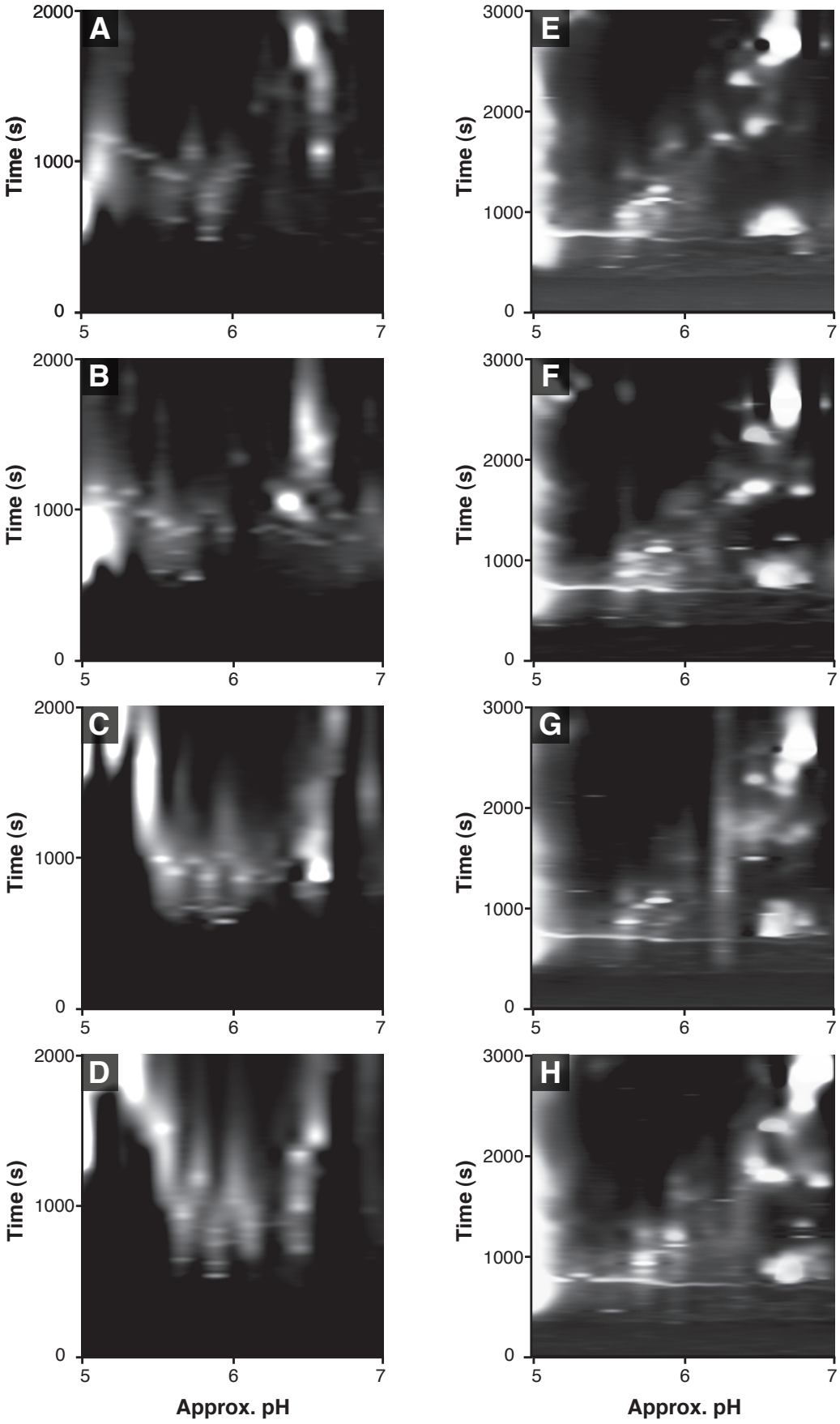
Figure S1. Results of 2nd-dimension separations of a low-mass protein ladder as detected with a rotary confocal fluorescence scanner. Electrophoresis at 200 V/cm was carried out under (A) native and (B, C) denaturing conditions achieved by adding 0.1% SDS to the run buffer, and are presented as pseudo-gel images. The chip in C was heated to 85°C after an initial electrophoresis period of 30 s. D–F presents single-channel traces from native, denaturing, and 85°C denaturing cases, respectively. Front and rear peaks in each of the denaturing traces are highlighted red, and the zone between them shaded grey.

Figure S2. Analysis of cell lysates by microDIGE illustrating run-to-run instability in the native second dimension separation where successive runs of cell lysates (A–D, 100 ng/μL) yield markedly different fluorescence patterns. The instability is eliminated by including 7 M urea in the 2nd-dimension separation matrix and adjusting the electric field to 150 V/cm, resulting in successive runs (E–H) with little variation in elution patterns.

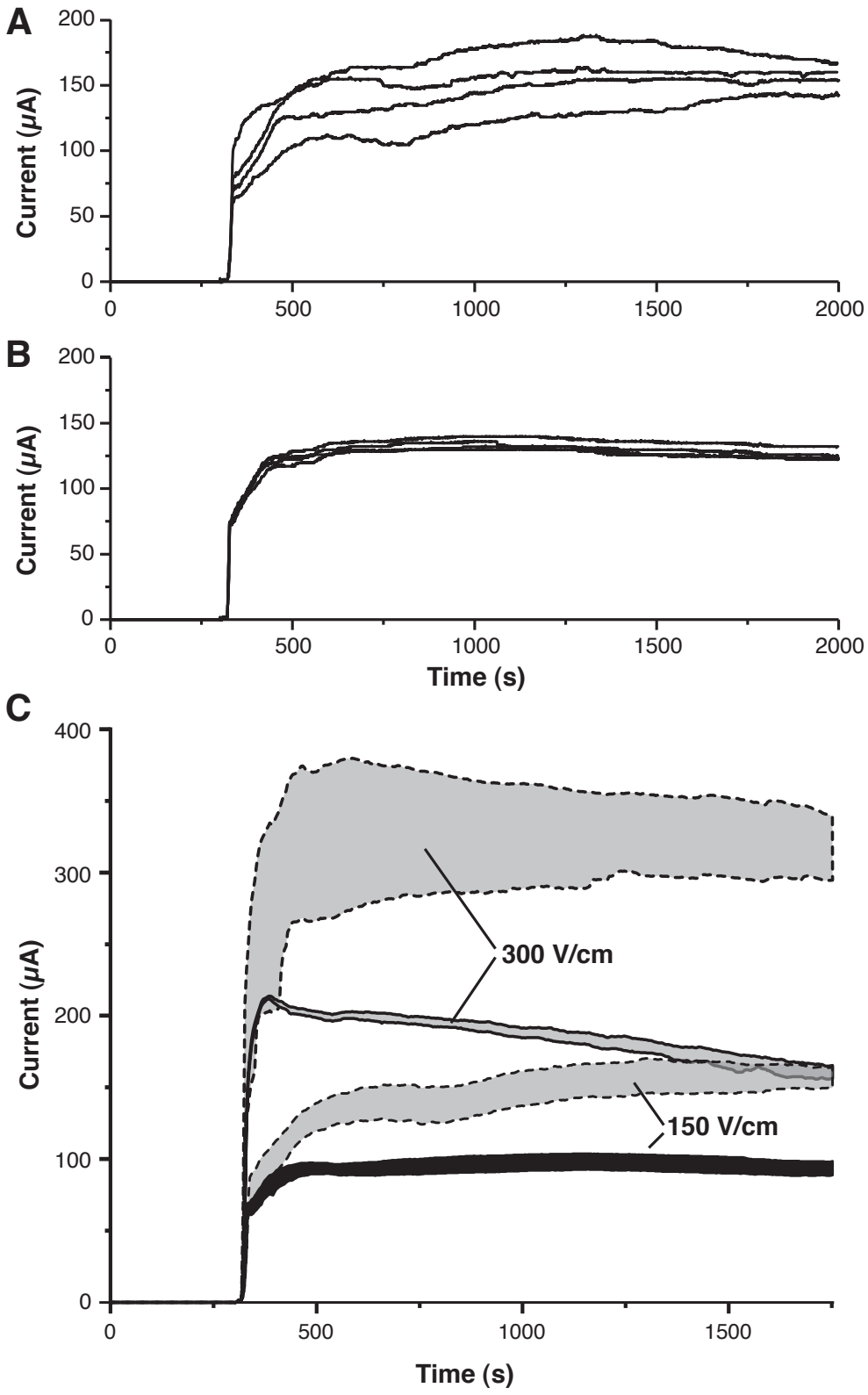
Figure S3. Electrophoresis currents measured during runs presented in Figure 5 indicate that current is an excellent predictor of stability in the 2DE elution pattern. Currents in (A) are taken from runs in Figure 5A–D. Currents in (B) are taken from runs in Figure 5E–H, which include 7M urea in the separation matrix. (C) The variation of 2nd-dimension electrophoresis current is sensitive to electric field and the presence of urea. Variation decreases as the field is decreased from 300 to 150 V/cm and is lower with urea (—) than without urea (---). Variance is minimized at 150 V/cm and with urea in the separation matrix (solid black).



Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3