Microfluidic Chemical Cytometry of Peptide Degradation in Single Drug-Treated Acute Myeloid Leukemia Cells

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S1. Single-cell electropherograms for untreated cells.

For untreated cells, peptide degradation was rapid and appeared to be complete, as only one peak, which co-migrated with K-FAM, was observed in most cases. However, out of 99 untreated single cells measured, two cells had a small second fragment peak (Figure S-1). These cells suggest that native peptide degradation rates are heterogeneous within the untreated population, but the process was too rapid compared to the pinocytic peptide loading protocol to fully characterize in intact, untreated cells.

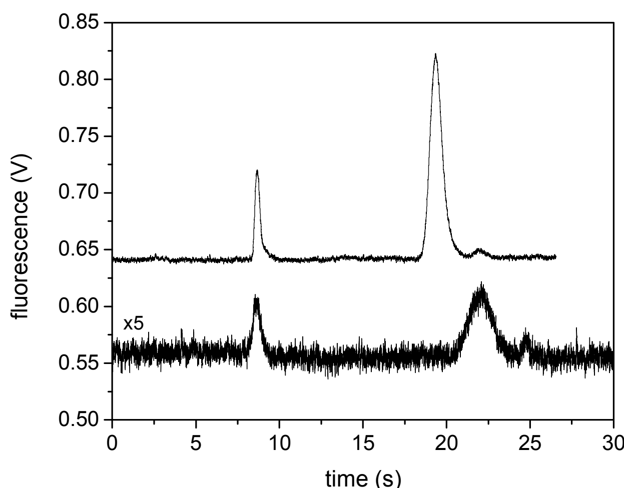

Figure S-1. Single-cell electropherograms of the two untreated cells that possessed a second fragment peak.

S2. Capillary electrophoresis.

Preliminary separations of ensemble lysate samples were performed on a commercial capillary electrophoresis system (PA-800, Beckman Coulter). The electrophoretic buffer was 140 mM borate, 70 mM SDS at pH 7.5 in an uncoated 30 µm i.d. capillary at an electric field strength of 600 V/cm and an effective length of 20 cm. Like the microchip separations, capillary separations of lysed, untreated cells showed only one fragment peak, which co-migrated with K-

FAM (Figure S-2a). In lysates prepared from drug-treated cells, the first fragment peak co-migrated with K-FAM, while none of the peaks co-migrated with the parent peptide (Figure S-2b). These capillary electrophoresis data support the microchip data in suggesting that K-FAM is a major component of one fragment peak and establish that intact parent peptide was not present even in drug-treated cells.

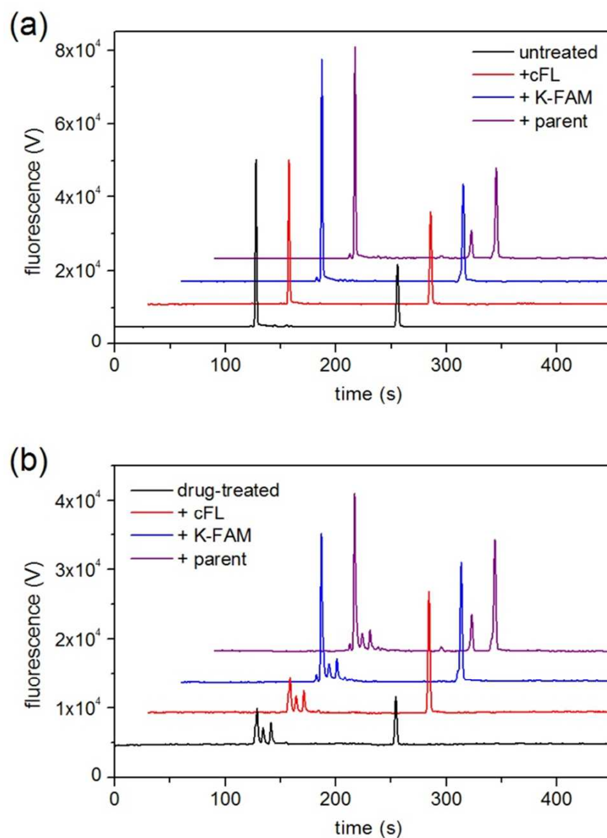

Figure S-2. Capillary electropherograms of ensemble lysate samples (black) for (a) untreated cells and (b) treated cells. Each lysate sample was successively spiked with standard solutions of cFL (red), K-FAM (blue), and the parent peptide (purple). Note that the migration order of K-FAM and cFL is reversed compared to the microchip separations, so that the cFL internal standard peak is the last peak (rather than the first peak) in each electropherogram.

S3. Heterogeneous fragment patterns for single cells lysed within a 15-min time period.

The throughput of the single-cell system averaged 37 ± 7 cells/h over 13 experiments. As a result, approximately 9 cells were measured during every 15-min period, as seen in Figure S-3 below. These data demonstrate that even cells measured closely in time could possess widely varying peak patterns, indicating that the heterogeneity observed was not due solely to differences in time elapsed since peptide loading. Single-cell electropherograms also revealed fragment peak patterns not observed in ensemble data. For example, the third trace from the bottom in Figure S-3 has two fragment peaks, a pattern not seen at any time point in the ensemble measurements (Figure 2a).

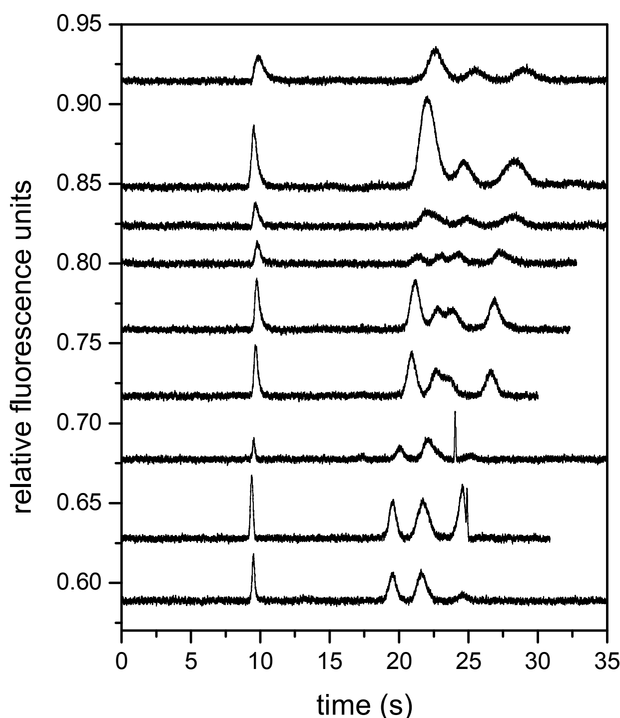

Figure S-3. Single-cell electropherograms for 9 cells lysed within a 15-min period 1.5 h after peptide loading. The first peak in each trace was the internal standard, carboxyfluorescein.

S4. Estimation of total cellular peptide concentration.

Gated injections of 1 nM, 10 nM, and 100 nM K-FAM standards were performed using a 20 µm wide, 20 µm deep simple cross chip. The length of the injected plug was determined using fluorescence microscopy, a CCD camera, and ImageJ software. Along with the channel width and depth, this value was used to determine the average injection volume of 58 ± 2 pL (n =5). The microdevice was then transferred to the microfluidic chemical cytometry detection system. For the three K-FAM concentration used, this corresponds to injections of 5.8×10^{-20} mol, 5.8×10^{-20} mol, 5.8×10^{-20} mol, respectively. A calibration curve of peak area vs. moles injected had an equation of $y = 1.8 \times 10^{-18}$ x and $R^2 = 0.963$. This calibration curve was used to estimate the moles of peptide present in the cells with the lowest (0.197 V*s) and highest (274 V*s) total fragment peak areas, resulting in limits of 4×10^{-19} mol and 5×10^{-16} mol.

S5. Safety considerations.

Proper precautions should be exercised when using a high voltage power supply to avoid electric shock. Eye protection should be used when aligning and operating lasers. Human cell lines require Biosafety Level 2 procedures, and proper personal protective equipment should be used when handling any chemotherapeutic drug, especially when dissolved in DMSO.